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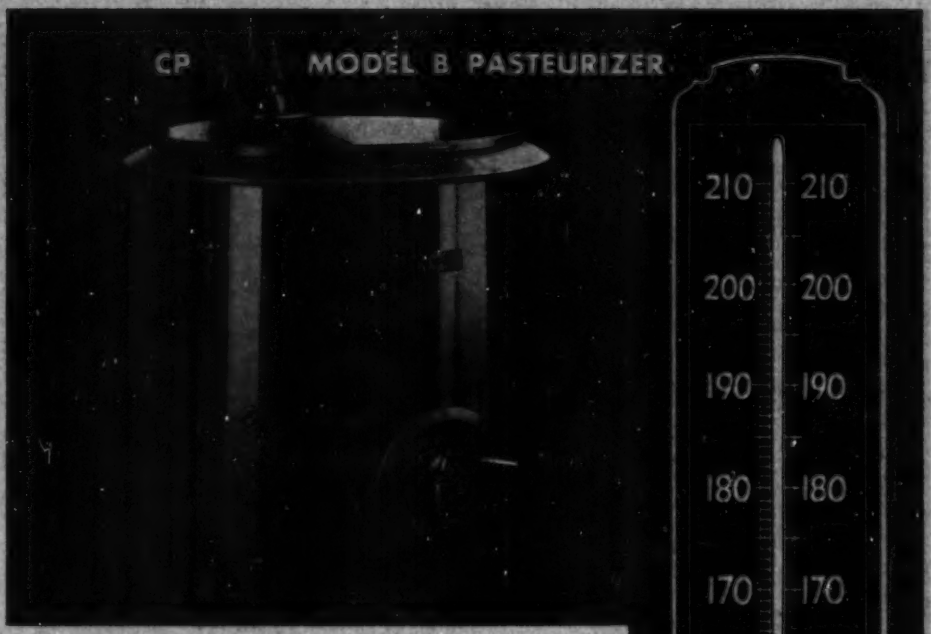

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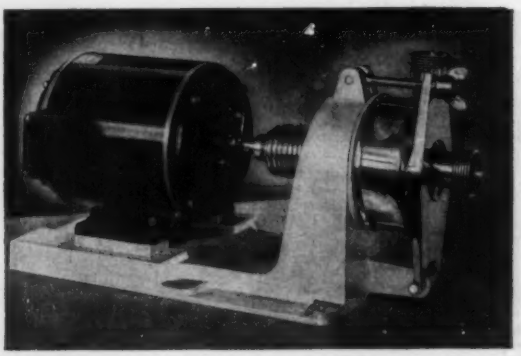

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JOURNAL OF DAIRY SCIENCE

VOLUME XX JANUARY, 1937 NUMBER 1

A STUDY OF THE SEVERAL MINNESOTA REAGENTS FOR THE DETERMINATION OF FAT IN BUTTERMILK*

E. W. BIRD AND D. F. BREAZEALE
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INTRODUCTION

To date, three Minnesota reagents for the determination of fat in dairy products have been presented. For convenience the original reagent (4) will be designated, Reagent A, that described by Sommer (5), Reagent B and the one sold by the Kimble-Nafis Company (2) during the spring of 1935, Reagent C.

Dr. B. L. Herrington called the attention of the writers to the fact that results obtained with Reagent C did not conform with the results they had reported for Reagent A. The results with Reagent C were said to be much lower than those for Reagent A. Greater saponification with Reagent C than with Reagent A was suggested as the cause of the difference although the methods or data on which this suggestion was based were not disclosed.

The objectives of this study were, therefore, to determine the magnitude of the difference between the tests obtained by Reagents A and C, to determine to what extent this difference resulted from greater saponification by the latter reagent and to determine the general relationships existing among the results obtained by the three reagents.

METHODS AND RESULTS

1. The effect of different manipulative methods on the results obtained with Reagent C. The effect of the use of phenolphthalein in 95 per cent alcoholic solution and the use of two 0.5 minute as against a 5, 2 and 1 minute centrifuging procedure were checked. Nine gm. samples, 10 cc. of reagent and 5 minute digestion times at approximately 90° C. were used. The results are presented in Table 1. They indicate that the use of the standard centrifuging procedure with 95 per cent alcoholic phenolphthalein yielded the highest test. It was considered that the method yielding the

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TABLE 1

Results obtained with Reagent C with different testing procedures on the same buttermilk sample. (Mojonnier test 0.574%)

TEST NUMBER	2–0.5 MIN. CENTRIFUGING PERIODS; 2 CC. 1% PHENOLPHTHALEIN IN 95% ALCOHOL	2–0.5 MIN. CENTRIFUGING PERIODS; NO PHENOLPHTHALEIN USED	5, 2 AND 1 MIN. CENTRIFUGING PERIODS; 2 CC. 1% PHENOLPHTHALEIN IN 95% ALCOHOL	5, 2 AND 1 MIN. CENTRIFUGING PERIODS; NO PHENOLPHTHALEIN USED
	Per cent	*Per cent*	*Per cent*	*Per cent*
1	0.10	0.06	0.22	0.16
2	0.10	0.06	0.22	0.16
3	0.10	0.07	0.22	0.16
4	0.09	0.06	0.23	0.16
Average	0.098	0.063	0.222	0.16

highest test would be the best one to use in the indirect method of determining saponification that will be described later.

2. A comparison of the results obtained with the three Minnesota reagents when 5, 2 and 1 minute centrifuging periods were employed. Here 9 gm. samples, 10 cc. reagent and a 6 to 7 minute digestion period at approximately 90° C. were used (4). The data are recorded in Table 2.

TABLE 2

Comparison of tests of the same buttermilk with Minnesota reagents A, B and C, with 5, 2, and 1 minute centrifuging periods. (Mojonnier test 0.574%)

TEST NUMBER	REAGENT A	REAGENT B	REAGENT C
	Per cent	*Per cent*	*Per cent*
1	0.36	0.30	0.22
2	0.38	0.31	0.22
3	0.42	0.29	0.23
4	0.38	0.30	0.22
5	0.39	0.26	0.27
6	0.40	0.28	0.26
7	0.41	0.28	0.26
8	0.44	0.28	0.24
9	0.40	0.26	0.24
10	0.41	0.27	0.26
11	0.38	0.26	0.24
12	0.40	0.26	0.22
13	0.41	0.24	0.21
14	0.41	0.24	0.20
15	0.41	0.24	0.19
Average	0.400	0.271	0.232

They indicate that when the procedure recommended originally for the Minnesota test is employed the fat test of the same buttermilk drops from Reagent A, to Reagent B, to Reagent C.

3. Methods employed to obtain data from which the degree of saponification with the three Minnesota reagents was estimated. For these runs 9 gm.

TABLE 3

Check on the saponification of butterfat in performing the Minnesota fat test in buttermilk with reagents A, B, and C. (Mojonnier test 0.736%)

HEATING TIME IN MINUTES	REAGENT A		REAGENT B		REAGENT C ; 95% ALCOHOL PHENOLPHTHALEIN 5, 2, 1 MIN. CENT.		REAGENT C ; AQUEOUS PHENOLPHTHALEIN 5, 2, 1 MIN. CENT.		REAGENT C ; 95% ALCOHOL PHENOLPHTHALEIN 2–½ MIN. CENT.	
	Test	% of max.	Test	% of max.	Test	% of max.	Test	% of max.	Test	% of max.
	0.03		0.03		0.02		0.01		0.01	
0.0	0.02		0.04		0.03		0.01		0.01	
	0.03		0.07		0.03		0.02		0.02	
Average	0.027	4.91	0.047	8.86	0.027	5.59	0.013	4.06	0.013	4.48
	0.22		0.37		0.40		0.21		0.12	
2.0	0.14		0.37		0.32		0.21		0.12	
	0.22		0.43		0.28		0.21		0.14	
Average	0.193	35.10	0.390	7.36	0.333	68.85	0.210	65.60	0.127	43.80
	0.58		0.53		0.47		0.32		0.23	
5.0	0.54		0.53		0.49		0.32		0.24	
	0.53		0.53		0.49		0.32		0.28	
Average	0.550	100.00	0.530	100.00	0.483	100.00	0.320	100.00	0.250	86.20
	0.53		0.47		0.37		0.29		0.31	
10.0	0.54		0.47		0.35		0.28		0.32	
	0.54		0.49		0.30		0.29		0.24	
Average	0.537	97.60	0.483	91.10	0.340	70.40	0.283	88.41	0.290	100.00
	0.54		0.46		0.26		0.18		0.18	
15.0	0.52		0.44		0.26		0.20		0.06	
	0.54		0.44		0.26		0.20		0.20	
Average	0.533	96.90	0.447	84.30	0.260	53.83	0.193	60.32	0.147	50.70
	0.49		0.36		0.16		0.16		0.13	
25.0	0.46		0.33		0.17		0.15		0.15	
	0.48		broken		0.17		0.17		0.15	
Average	0.477	86.70	0.345	65.10	0.167	34.58	0.160	50.00	0.143	49.30
	0.48		0.26		0.12		0.05		0.09	
40.0	0.50		0.25		0.09		0.06		0.01	
	0.47		0.24		0.00		0.06		0.12	
Average	0.483	87.50	0.250	47.15	0.070	14.49	0.056	17.48	0.073	25.16
	0.51		0.18		0.15		0.06		0.13	
60.0	0.54		0.17		0.14		0.06		0.12	
	0.54		0.20		0.00		0.06		0.16	
Average	0.530	96.40	0.183	34.52	0.097	20.08	0.060	18.75	0.137	47.25

samples, 10 cc. of reagent and a bath temperature of approximately 90° C. were employed. Three manipulative methods were employed with Reagent C. These are given in Table 3. The aqueous phenolphthalein was prepared by dissolving the indicator in N/10 NaOH. The digestion of the samples was carried out for various lengths of time, and except where otherwise stated, 5, 2 and 1 minute centrifuging periods were employed. One large, well-mixed buttermilk sample was used for all the tests. The data are presented in Table 3.

4. It was considered desirable to obtain data comparing tests with the three Minnesota reagents, the American Association, the Babcock and the Mojonnier testing methods. These data were again obtained with one large, well-mixed buttermilk sample. The methods employed for all but the tests with Reagent C have been previously described (1). In determining the fat percentage with Reagent C, 9 gm. samples, 10 cc. of reagent, 2 cc. of 1 per cent phenolphthalein in 95 per cent alcohol, 5 minute digestion periods at approximately 90° C. and two 0.5 minute centrifuging periods were used (3). The data are presented in Table 4.

TABLE 4

Comparison of tests of the same buttermilk with Minnesota reagents A, B, and C, the Babcock and the American Association methods. (Mojonnier test 0.745%)

TEST NUMBER	MINNESOTA REAGENT A	MINNESOTA REAGENT B	MINNESOTA REAGENT C	AMERICAN ASSOCIATION	BABCOCK
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	0.54	0.50	0.26	0.74	0.30
2	0.52	0.40	0.23	0.70	0.20
3	0.52	0.46	0.16	0.78	0.24
4	0.48	0.46	0.16	0.72	0.17
5	0.53	0.47	0.16	0.72	0.25
6	0.49	0.49	0.21	0.74	0.17
7	0.52	0.47	0.17	0.76	0.22
8	0.50	0.48	0.19	0.72	0.30
9	0.55	0.44	0.16	0.76	0.21
10	0.51	0.47	0.18	0.72	0.27
11	0.51	0.46	0.26	0.76	0.27
12	0.47	0.48	0.24	0.75	0.20
Average	0.512	0.465	0.198	0.739	0.233

DISCUSSION OF RESULTS

The data of Table 1 show that when 5, 2 and 1 minute centrifuging periods were used with Reagent C, the resulting tests were higher than with the other methods, providing a 1 per cent phenolphthalein solution in 95 per cent alcohol was used. For this reason, and because of the fact that this centrifuging procedure is like that for Reagents A and B, it was adopted for the study of the amount of saponification that occurs during the digestion of the samples.

It is evident from Table 2 that when this centrifuging method is employed with all three reagents the results obtained vary considerably and become progressively lower from Reagent A, to Reagent B, to Reagent C.

Fig. 1 presents the averages of the triplicate tests for Reagents A, B and C (alcoholic phenolphthalein) of Table 3, plotted against digestion time. Graphs were drawn through the points representing each reagent. These

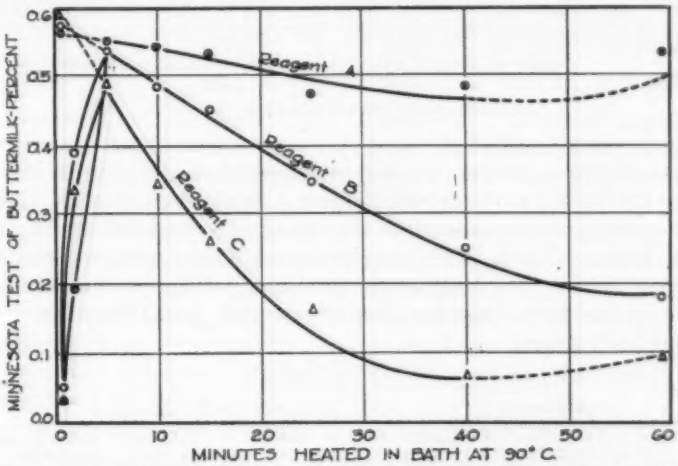

FIG. 1. The relationship between the digestion time and fat test with Minnesota reagents A, B and C.

rose steeply to maxima at approximately five minutes digestion time. As the time lengthened the curves dropped off; that for Reagent A, least, that for Reagent C, most and that for Reagent B, at an intermediate rate. The portions of the curves to the right of the maxima were extrapolated to zero time. This value it was considered should have given the maximum tests that it would have been possible to have obtained with these reagents had no saponification occurred.

The method may be criticized because of the fact that the values obtained by extrapolation of these curves may not be as precise as might be desired. Considering that Reagent C contains "castor oil potassium hydroxide soap solution" (2) the usual methods of acidification and extraction could not be carried out. The method employed seemed to be the logical one for determining the amounts of saponification under these conditions. The extrapolated values obtained for the three reagents were:

Reagent A	0.566 per cent
Reagent B	0.576 " "
Reagent C	0.598 " "
Average	0.580 per cent

These values fall surprisingly closely together considering the crudeness of the method employed.

If the assumption is made that the average of the above values (0.580 per cent) is the value that would have been obtained had no saponification occurred, the percentages of saponification if the tests were maximum are:

	VALUE OF MAX. TEST	0.580—MAX. TEST	% TEST LOWERED BY SAPONIFICATION
Reagent A	0.550	0.030	5.17
Reagent B	0.530	0.050	8.62
Reagent C	0.483	0.097	16.73

If the additional assumption be made, that the rate of saponification is the same for Table 4 as it was with Reagent A for Table 3, then the Table 4 reading corrected for saponification, the difference between this reading and that for Reagent C with two 0.5 minute centrifuging periods and the percentages of the corrected reading equivalent to this difference, the difference caused by saponification and the difference resulting from different manipulations of the tests are:

Data from table 4

TEST AS READ REAGENT A	TEST CORR. FOR SAPONIFICATION REAGENT A	TEST AS READ REAGENT C	TOTAL DIFF. AS % CORR. TEST REAGENT A	% CORR. TEST (A) LOWERED BECAUSE OF SAPONIFICATION	% CORR. TEST (A) LOWERED BY DIFF. IN MANIPULATION OF TEST
0.512	0.534	0.198	62.23	16.73	45.50

When the following data from Tables 1 and 2 are employed: a. Reagent C, 5, 2 and 1 minute centrifuging periods = 0.232 per cent, b. Reagent C, two 0.5 minute centrifuging periods = 0.098 per cent and, c. test with Reagent A corrected for saponification = 0.422 per cent, the percentages of the corrected Reagent A test equivalent to; d. the total difference (c–b), e. that resulting from manipulative difference (a–b) and f. that resulting from saponification (d–e) are respectively 45.02, 31.72 and 13.30 per cent.

At first glance these two sets of results (calculated from Table 4 and from Tables 1 and 2) seem to present considerable variation. If, however, the percentages of the total difference between the corrected Reagent A and the two, 0.5 minute centrifuged Reagent C readings, that is caused by saponification and by the manipulation of the test are calculated, it is found that very close agreement exists between the two sets of data:

DATA FROM	PERCENTAGE TOTAL DIFF. ⌭ SAPONIFICATION	PERCENTAGE TOTAL DIFF. ⌭ MANIPULATION
Table 4	26.89	73.11
Tables 1 and 2	29.33	70.47

The data of Table 4 indicate that the values for buttermilk fat content obtained with the commercial reagent (C), used as directed (3), with two 0.5 minute centrifuging periods yield results that approximate very closely the regular Babcock method for buttermilk testing.

The last two columns of Table 3 indicate that the alcohol present in a 1 per cent solution of phenolphthalein in 95 per cent alcohol increases the saponification rate after the maximum test is reached, that it lowers the maximum test obtained and that it retards the attainment of the maximum test from the 5 minute to the 10 minute digestion point.

SUMMARY AND CONCLUSIONS

1. Different values for the fat content of buttermilk are obtained with each of the three Minnesota reagents that have been proposed, when the same sample of buttermilk is tested.

2. The commercial reagent (C) yields a test slightly lower than that obtained by the regular Babcock test, when Reagent C is used in accordance with the directions furnished with the reagent.

3. Saponification was shown to occur with all three reagents. The amount of saponification for these reagents was estimated as follows: Reagent A, 5.17 per cent, Reagent B, 8.62 per cent and Reagent C, 16.73 per cent.

4. Approximately 70.0 per cent of the difference between the reading with Reagent A (corrected for saponification) and that of Reagent C is the result of differences in manipulative procedure; the other 30 per cent is caused by saponification.

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VARIATIONS IN THE VITAMIN C CONTENT OF MILK*

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INTRODUCTION

In previous publications (1, 2) attention has been called to the fact that the chemically determinable vitamin C content of milk may vary greatly from day to day, and between individual cows. It has also been found (3) that under suitable conditions milk may be kept 24 hours or more without serious loss of vitamin C.

In the fall of 1935 a series of tests was started, on individual cows from the station dairy herd, to determine variations in the vitamin C content of milk. These tests were designed to find differences due to the hour, day, and season when the test was made; and also variations due to the breed, individuality, milk yield, age, and stage of lactation of the cow. Other workers (4) have discussed some of these factors, and some of their findings differ from those of this station.

PROCEDURE

Forty cows about equally distributed among the Ayrshire, Guernsey, Holstein, and Jersey breeds were tested 3 consecutive mornings in October and again in November. Sixteen of these cows were also tested both morning and evening for 7 days. Subsequent tests included all 55 to 58 cows of the station herd, distributed about equally among the four breeds. In these tests 10 ml. of milk stood 30 minutes with 15 ml. of 10 per cent trichloracetic acid. Five ml. of the resulting serum was titrated with 2–6-dichlorophenol-indophenol (4, 5). Except as noted below all cows were fed, according to production, a ration consisting of alfalfa hay, atlas sorgo silage, and a grain mixture. No pasture was available to these cows.

As a part of a study of vitamin C metabolism, which will be reported in detail elsewhere, 3 cows were fed after the April tests as much green rye as they would consume. By the end of one week they were each consuming approximately 65 Kg. per day of rye that tested 0.86 mg. of vitamin C per gram. This would be equivalent in vitamin C content to 30 gallons of orange juice per cow per day. The vitamin C contents of the milk and urine were determined at suitable intervals. Milk samples from 3 other cows previously found to give milk of comparable vitamin C content and constancy were also tested at the same milkings as the test animals.

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* Contribution No. 211, Department of Chemistry, and Contribution No. 108, Department of Dairy Husbandry.

RESULTS

It was soon recognized that any value for vitamin C is influenced by several factors. In October, 107 comparisons on cows milked at 12-hour intervals indicated that evening milk contains 2.0 ± 0.7 mg. per liter more vitamin C than morning milk, with less than 1 chance per million that the difference was all accidental. This difference may be helpful in finally locating the mechanism by which vitamin C secretion is controlled. It also indicates the necessity for making comparative tests at the same time of day.

Each monthly test provided 2 consecutive comparisons of daily changes. Daily changes in the average value of the herd are shown in Table 1.

TABLE 1

Daily change of vitamin C in herd milk

	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	APR.
Day	Change in vitman C content, mg. per liter						
1st.–2d.	+4.5	+0.3	−4.4	+6.1	+3.1	+3.2	+2.2
2d.–3d.	−5.8	+2.4	+1.2	+0.6	−0.1	+0.5	−1.7

These variations are large enough to indicate that the vitamin C content of milk from a given cow or herd can not be adequately determined by a single test.

Thirty-six cows beyond the second month of lactation were tested both in December and in February. The coefficient of correlation between these tests was .89. The corresponding correlation between values in February and March was .66. The 6 correlations calculated ranged from .66 to .94. These values are high and justify the conclusion that comparisons between individuals or groups, at the same time, are more reliable than the single values compared, or than comparisons of values determined at different times.

The simplest measure of the effect of season is the herd average for each month. As will be shown later, the early stages of lactation coincide with a low vitamin C content. The average for the herd excluding cows in the first and second months of lactation was therefore calculated. Both these sets of values and the ratio of each value to that for the preceding month are shown in Table 2.

TABLE 2

Monthly average vitamin C content of milk from whole herd and herd excluding fresh cows

	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	APR.
	Whole Herd						
No. cows	40	40	58	57	55	55	58
C mg./liter	23.6	27.2	29.2	23.8	22.2	26.0	24.5
Ratio to previous month		1.14	1.07	.81	.94	1.17	.94
	Herd with 1st and 2nd months lactation excluded						
No. cows	34	35	47	48	47	43	47
C mg./liter	23.6	27.0	29.7	24.2	22.8	26.4	25.3
Ratio to previous month		1.14	1.10	.81	.94	1.16	.96

The effect of excluding cows in the first 2 months of lactation is small. This is the largest known secondary factor which was not held constant during the comparison of different seasons. It seems reasonable therefore to conclude that the changes for the reduced herd are related to seasonal changes. The average vitamin C content of milk from all cows and for all months tested was 25.5 mg. per liter.

The low values in January and February coincide with the season of continued wintry weather. For these months the cows were stabled most of the time. The temperatures in the barn approximated the out-door temperatures to which the cows were exposed in December and March. The humidity in the barn was higher. The cows of course received less exercise and sunshine when stabled. Since the causes of the seasonal changes in vitamin C are obscure, it is impossible to predict when similar changes will be found again. Statistical analysis indicates, however, that there is less than 1 chance per million that the rise from October to December, the fall from December to February, or the rise from February to March is accidental.

For the 3 cows fed green rye, during some 12-hour interval within 60 hours after rye was first fed, the output of vitamin C in urine compared to the average of 2 similar intervals just before feeding rye increased 7.0, 5.1, and 4.5 fold respectively for the 3 cows. This included increases in concentration of 4.3, 1.6, and 2.0 fold respectively. The effect of green rye feeding on vitamin C content of milk is summarized in Table 3.

TABLE 3

Vitamin C in milk before and after feeding green rye

VITAMIN C EXPRESSED AS PER CENT OF CONTROLS

Milkings	Cinderella	Gaiety	Trixie
1–4 before	99	107	108
2–5 after	108	107	107
11–16 after	118	108	106
30	101	90	106

An increased output of vitamin C in urine is recognized evidence that the body reserves of the cows were normal. Whether or not the observed small increase in the milk of the first cow was significant cannot be stated. In the case of the other 2 cows, the increased intake of vitamin C was unquestionably accompanied by an increased excretion in the urine but no increased secretion in the milk. Fifteen days after the initial heavy rye feeding there was no significant increase in the vitamin C concentration of the milk from any of the 3 cows.

If cows beyond the second month of lactation were grouped by breeds, Jerseys were highest in vitamin C content in all months. No other breed held a constant rank. The average values were: Ayrshires 24.1, Guernseys 26.0, Holsteins 23.5, and Jerseys 29.2 milligrams per liter. There is no doubt but the higher values for this particular Jersey herd are significant. How-

ever, one day when this Jersey herd averaged 33.5 and the whole station herd averaged 28.5, another Jersey herd of 10 individuals located near the station and receiving a somewhat similar ration averaged only 24.4. In the station herd the average for Jerseys was 1.24 times that for Holsteins. Of the 8 Jerseys whose milk was richest in vitamin C, 6 were genetically related. The number of animals involved is too small to justify conclusions regarding heredity as a factor in vitamin C production. The genetic relationship between the high cows of this herd, together with the low value recorded for the

TABLE 4

Vitamin C in milk as ratio to previous month: actual (A), and relative to herd with 1st two months excluded (R), for numbers of cows (N)

STAGE OF LACT.		NOV.	DEC.	JAN.	FEB.	MAR.	APR.	AVE. R	TOTAL N
	A	1.29	1.29	.91	.97	1.59	1.02		
2/1	N	4	1	4	5	3	9		26
	R	1.13	1.15	1.12	1.03	1.37	1.06	1.10	
	A	1.30	1.08	.84	.94	1.32	.89		
3/2	N	2	4	6	4	2	3		21
	R	1.14	.98	1.04	1.00	1.14	.92	1.02	
	A	1.19	1.12	.82	.84	1.33	.92		
4/3	N	1	2	7	6	4	2		22
	R	1.05	1.02	1.01	1.00	1.15	.96	1.03	
	A		1.11	.79	.96	1.20	.89		
5/4	N		10	4	7	5	4		30
	R		1.01	.98	1.02	1.03	.93	1.00	
	A	1.19		.84	.88	1.14	.92		
6/5	N	1		11	4	5	5		26
	R	1.05		1.03	.93	.98	.96	.99	
	A	1.11	1.04		.91	1.28	.90		
7/6	N	9	1		11	4	6		31
	R	.98	.96		.97	1.10	.94	.98	
	A	1.12	1.08	.74		1.19	.90		
8/7	N	3	9	1		11	4		28
	R	.98	.98	.91		1.02	.94	.99	
	A	1.05	1.12	.80	.92		.96		
9/8	N	3	3	9	1		11		27
	R	.92	1.02	.99	.98		1.00	.99	
	A	1.21	1.11	.83	.95	1.25			
10/9	N	2	3	3	8	1			17
	R	1.06	1.01	1.02	1.01	1.08		1.02	
	A	1.13	1.01	.72	1.00	1.22	.78		
11/10	N	1	2	1	2	6	1		13
	R	.99	.92	.89	1.06	1.05	.81	1.00	

second herd mentioned above does, however, cast some doubt on the generally high values for Jerseys indicated by this herd.

The ratios of high to low vitamin C producing cows in each breed, averaged for 7 months, were: Ayrshires 1.54, Guernseys 1.27, Holsteins 1.41, and Jerseys 1.48. The smallest ratio between individuals within a breed was larger than the largest ratio found between breeds.

There appears to be no simple relation between vitamin C content and yield of milk. This conclusion is based both on comparisons of average values and also on comparisons of changes from day to day. An apparent decided trend in comparisons made 1 month may be reversed 1 or 2 months later.

The relation of vitamin C content of milk to the age of the cow is similar to that which has been established for fat content. It is small and unimportant.

To make use of a sufficient number of cows from the station herd for a valid statistical study of the effect of stage of lactation on the vitamin C content of milk, comparisons must include more than 1 month's test. Therefore, the effect of season as well as the effect of the individual cow, as a producer of milk of high or low vitaman C content, must be eliminated. The individuality factor can be eliminated by comparing changes in vitamin C content rather than actual content. The effect of season can be approximately eliminated by comparing changes in cows at a particular stage of lactation with average changes in the whole herd. This approximation can be improved by eliminating from the average for the herd, cows in those stages of lactation where most change was thus indicated. Actual ratios of values for successive months, and also the relative ratios obtained by dividing each actual ratio by the actual ratio for all cows beyond the second month of lactation, are shown in Table 4.

The data in the "average" column in Table 4 indicate that cows in the first month of lactation produce milk with about 10 per cent lower vitamin C content than in later months. Later stages of lactation do not influence the vitamin C content of milk.

CONCLUSIONS

The season of the year, the individuality and breed of the cow, and the stage of lactation appear to be the most important factors causing variations in the vitamin C content of fresh milk from well fed cows.

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A STUDY OF THE CHURN CLEANING METHODS USED BY PLANTS PRODUCING BUTTER OF VARIOUS YEAST AND MOLD COUNTS[1]

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The yeast and mold count of butter is commonly considered to give some indication of the sanitary efficiency of a creamery. A high yeast and mold count may be due to a number of factors; such as, inefficient pasteurization of the cream, contaminated butter cultures, infected churns, impure wash-water, a low salt content in the butter, careless handling of the butter after churning, infected wrappers and containers, and others. Practical experience as well as scientific investigations, however, have indicated that the improper care of the churn is one of the principal causes of high yeast and mold counts, because the churn being generally constructed of wood, is difficult to sterilize, especially if the rollers, shelves, or other parts of the churn have developed crevices and if organic matter has been allowed to accumulate in these as they most certainly will.

Since yeast and mold counts have been made regularly for nearly three years on all butter samples entered in the educational scorings conducted monthly by the State College of Washington, it was decided to examine the churn cleaning methods employed by the participating creameries and to study the extent to which these methods are reflected in the yeast and mold counts and perhaps the scores of the resulting butter. Consistently low yeast and mold counts should indicate satisfactory methods of caring for the churns.

REVIEW OF LITERATURE

That the churn is one of the greatest sources of contamination of butter made from pasteurized cream has been shown early by many investigators. Lund (1) found that high yeast counts were caused nine out of ten times by infected churns. Macy and Combs (2) discovered that the churn was the source of molds in 65 per cent of the creameries studied, salt in 33 per cent, the starter in 40 per cent, the water in 44 per cent and pipes and pumps in nearly 75 per cent of the creameries. Mold spores were carried by 90 per cent of the dry parchment and cloth circles examined. Macy, Coulter and Combs (3) found the churn to be the most prolific of all sources of yeast and mold infection. They frequently found a higher yeast and mold count in the butter than in the cream and the buttermilk.

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The difficulty of eliminating yeasts and molds from the churn is explained by such findings as those of Macy, Combs and Morrison (4) who established the presence of molds in the joints between the churn staves at a depth of ¾–1 inch. Libbert (5) found that yeasts and molds will grow well even on churn wood alone.

Various procedures for cleaning and sterilizing the churn have been advocated. Hunziker (6) recommends as a daily treatment for churns to rinse with 50 gallons of hot water containing some suitable alkali, to drain the churn and then revolve it for at least 30 minutes with workers in gear while at least one quarter full of water at 200° F. or over. The churn should then be drained thoroughly and left to dry with the doors up and open. Before use on the next day the churn should be rinsed with cold water. He says that chemical disinfectants seem to be incapable of penetrating the wood adequately.

Other investigators including Gregory (7), Lund (8), Hood and White (9), Zakariasen (10) have shown the necessity of using a large volume of water at a high temperature for a sufficient length of time together with some alkali to keep the churn in a sweet sanitary condition. Occasional liming of the churn is often recommended to destroy yeast and molds effectively. Hood and White (9) advise the liming method of Lund and state that liming tends to harden the wood of the churn. Gregory (7) recommends rinsing the churn just before use with a chlorine germicide or washing again with water at 180–190° F. for 5 minutes and then rinsing with cold water.

Other investigators such as Shutt (11), James (12) and Olson and Hammer (13) obtained favorable results using chemical sterilizer solutions, especially when preceded by a satisfactory hot water treatment. James (12), however, found the evidence of the effectiveness of chemical sterilizers inconclusive and recommends the hot water treatment with ample periods of exposure and using enough water to cover the rollers and emphasizing the importance of working the rollers in the washwater to kill the organisms released by the strain of working on the churn parts. Morrison, Macy and Combs (14) found all chlorine sterilizers including sodium hypochlorite, alkaline crystalline hypochlorite, and chloramine T ineffective when used as the sole treatment. They demonstrated that the surface molds were destroyed by filling the churn one-third full with water at 96° C. (205° F.) and revolving one-half hour, but that the heat did not penetrate sufficiently to destroy molds underneath the surface of the wood. These authors considered alkaline washing solutions undesirable because of their softening effect on the wood and the use of steam was not recommended because of the blistering of the churn paint. They concluded that the most satisfactory treatment was the use of high temperature water for a sufficient period of time and repeated daily. Olson and Hammer (13) after an extensive investigation, however,

concluded that the deleterious effect of heat, alkali and chlorine compounds on the wood of churns should not be used as an argument against the proper treatment of churns, because in carelessly treated churns there will be serious deterioration of the wood due to the direct action of microorganisms. They found that churns contain comparatively small numbers of organisms, if rinsed well with 100–120° F. water, then washed carefully with ample amounts of water at 170–180° F. containing one pound of soda ash per 100 gallons, and finally revolved for 15–20 minutes while ½ full of water at 180–210° F. The use once a week of one pound of slaked lime per 100 gallons of the final rinse water was recommended. Following the above hot water treatment of 600 pound churns by a rinse with 10 gallons of sodium hypochlorite, chlorinated lime, or calcium hypochlorite in concentrations of 50–120 p.p.m. available chlorine at temperatures of 70–140° F. and revolving the churns containing these solutions for 5–45 minutes, caused still further reductions in the number of organisms in the churns. Treatment of the churn with cold saturated sodium chloride solutions after the regular hot water treatment did not significantly affect the number of organisms present.

Olson and Hammer (13) also showed that highly contaminated churns lowered the keeping quality of the butter when the butter was unsalted and was held at 32° or 45° F. The effect was insignificant when the butter contained 2.5 per cent salt. To what extent this effect of unclean churns on the quality of the salted butter is due to the direct action of yeasts and molds is still questionable.

Macy and Richie (15) found that with fresh butter there was no consistent and direct relationship between yeast and mold count and the score of the butter. They found a tendency, however, toward improved keeping quality for samples of low yeast and mold count, even though individual counts were no reliable indices of keeping quality. Interesting, although indirect, relationships have been pointed out by other investigators. Shutt (16) reports a considerably lower keeping quality for butter high in yeasts and molds than for butter of low counts when stored for six months at 10° F. Parfitt (17) noticed a tendency toward lower scores with higher yeast and mold, and especially yeast counts. Thomsen (18) found a definite trend toward reduced keeping quality with increasing yeast and mold counts. Ause and Macy (19) concluded that *Oospora lactis* utilizes or destroys the substances responsible for the pleasing starter flavor and aroma in butter.

PROCEDURE

Seventeen of the Washington plant operators participating in the monthly educational butter scorings reported the methods used by them in caring for their churns. Two one-pound butter samples from these plants had been received at 14–28 scorings and had been examined each time for yeasts and molds. All yeast and mold counts were made by plating 1 cc.

of a 1 : 10 dilution of the carefully sampled butter on Bacto Dehydrated Malt Agar adjusted to a pH of 3.5 and incubating at 21° C. (70° F.) for 5 days. The methods recommended by the American Dairy Science Association Committee on Bacteriological Methods (20) were followed in the procedure. The butter was scored by four competent judges when fresh and after one month in storage at 36–40° F. The identity of the samples was not known to the analysts and the judges.

For each of the above seventeen plants the median yeast and mold count of all samples submitted up to the time they reported their churn cleaning methods were calculated, and the distribution of high and low counts was studied.

RESULTS

Table 1 shows the ranking of the plants according to the median yeast and mold count of their butter entries.

TABLE 1

Yeast and mold counts of butter from plants reporting their churn cleaning methods

NO. OF CREAMERY	NUMBER OF SAMPLES ENTERED	MEDIAN YEAST AND MOLD COUNT PER ML.	% OF SAMPLES WITH YEAST AND MOLD COUNTS PER ML. OF				AVERAGE % SALT IN BUTTER
			1–50	51–100	101–1000	over 1000	
23	28	17.5	67.9	3.6	25.0	3.6	2.27
24	15	20.0	73.3	6.7	13.3	6.7	2.08
11	20	22.5	90.0	5.0	5.0	0	2.55
14	28	30.0	78.6	10.7	3.6	7.1	2.29
4	20	35.0	65.0	30.0	5.0	0	2.82
19	24	40.0	62.5	21.5	20.8	4.2	2.36
21	27	40.0	51.9	22.2	25.9	0	2.48
5	20	72.5	50.0	5.0	25.0	20.0	2.13
3	23	80.0	39.1	26.1	21.7	13.0	2.06
13	18	90.0	44.4	5.6	33.3	16.7	2.29
20	19	90.0	26.3	31.6	42.1	0	2.28
16	20	95.0	35.0	25.0	40.0	0	2.20
1	27	110.0	5.9	41.2	52.9	0	2.06
18	22	215.0	22.7	13.6	27.3	36.4	1.96
22	16	287.5	12.5	18.8	56.3	12.5	2.46
12	14	377.5	14.3	14.3	57.1	14.3	1.98
9	15	445.0	0	20.0	53.3	26.7	2.31

It will be seen that rather outstanding results were obtained by the first seven plants on the list. The median count of these plants for 15–28 months was below 50, and over 70 per cent of the entries of each of these plants showed counts of 100 or less.

In the case of three of these plants over 70 per cent of the entries had yeast and mold counts of 50 or less, and for the other four plants over 50 per cent of the entries showed counts of 50 or less. Although plant No. 23 ranked

highest in the median yeast and mold counts produced, plant No. 11 shows a fine record because it produced counts of 50 or less on 90 per cent of its samples. Its maximum count was 130, indicating very efficient care and consistent results. Plants No. 4 and No. 21 also had no counts exceeding 1000, their highest counts being 110 and 290 respectively. The performance of these seven plants appears to be distinctly superior to that of the other ten plants.

Because low counts can never be obtained without scrupulous care of the churn, the methods used by these plants must be considered and they are given by the operators as follows:

Creamery No. 23

Rinse with water at 120–130° F.
Wash with 150 gals. of water above 150° F. containing 1½–2 lbs. washing powder, revolving churn in high gear for 15 minutes.
Drain.
Leave doors up and open.
Before next use: Rinse with 100 gals. of cold water.

Creamery No. 24

Rinse with 75 gals. of water at 120° F., revolving churn 5 min.
Drain.
Wash with 150 gals. of water at 170° F. containing 2 lbs. of strained hydrated lime, revolving churn in high gear for 15–20 minutes.
Drain.
Rinse with 75–100 gals. of water as hot as possible (190° F.) revolving churn 5 minutes.
Drain.
Dry with doors up.

Creamery No. 11

Wash with 70–80 gals. of water at 120° F. containing 1 pound alkaline crystalline hypochlorite, revolving churn in high gear 5–6 minutes.
Slush out by revolving churn with doors off.
Drain.
Slush out with 80–90 gals. of water at 180–190° F. revolving churn 5–6 minutes.
Drain. Leave doors off with opening up.
Before next use: Rinse with 60–70 gals. water containing one-fourth of a pound of alkaline crystalline hypochlorite.

Creamery No. 14

Wash with lots of very hot water—no washing powder.
Drain.
Leave doors open and up.
Wash once a week with lime solution.

Creamery No. 4

Wash with 100 gals. of water at 100–120° F. (Alkaline crystalline hypochlorite added once a week.)
Wash with 75 gals of water at 180–200° F.
Drain.
Leave doors up and open.
Before next use: Rinse with cold water.

Creamery No. 19

Rinse with water at 170–180° F.
Wash with 150–160 gals. of very hot water in high gear for 20 minutes. Every two weeks add 4 pounds of alkaline crystalline hypochlorite.
Drain.
Doors down 10 minutes, then up.

Creamery No. 21

When churn is used daily:
Wash with 200 gals. of water at 125–140° F. running the churn 5 minutes in low gear and 10 minutes in high gear.
Wash with 200 gals. of water at 190–200° F. running the churn 5 minutes in low gear and 10 minutes in high gear.

When churn is not used daily:

Wash with 200 gals. of water at 135–140° F. running the churn 5 minutes in low gear and 10 minutes in high gear.
Wash with 200 gals. of water at 190–200° F. containing 1½ lb. dehydrated lime, running the churn 5 minutes in low gear and 10 minutes in high gear.

In either case:

Empty the churn by revolving in high gear.
Drain 10 minutes with doors down.
Leave with doors up, covered with a screen.

In looking over the methods used by these plants, it becomes apparent that either very hot water of a temperature of 190–200° F. or large amounts of hot water and alkali, or hot water and a chemical sterilizer were used in washing the churn. Such treatments will leave the churn dry and sweet. It is interesting to note that plant No. 11, which is a plant receiving a poor quality cream, and which in spite of that had the highest percentage of low yeast and mold counts, is the only plant in the group using a **chemical sterilizer** in the rinse water applied just previous to churning. Such a treatment or rewashing with very hot water was found necessary to secure low counts by Gregory (7). Most plants used **alkali** in some form in the washwater. Liming, at least occasionally, is practiced by some of these plants. The rather low temperature of 150° F. used by plant No. 23 is no doubt compensated for by the large amounts of water and the addition of about 0.15 per cent alkali.

As seen from table 1, the butter made by plants 4 and 11 is slightly higher

in salt content than that of the other plants. This slightly higher salt in brine concentration, however, would not be expected to decrease the yeast and mold counts materially during the time interval between manufacture and analysis of the butter. This was shown by Macy (21) who found that there was no greater tendency for the more highly salted butter samples to decrease in yeast and mold count than for those of a lower salt concentration, after the butter had been in storage a week at 30–45° F. and in transit for a week at 42–67° F. Similar results were obtained by Macy, Coulter and Combs (3). Thus the slightly higher salt concentration in the butter of plants 4 and 11 would not be sufficient to produce the low yeast and mold counts, if the butter had been heavily contaminated from the churn.

While low counts must indicate satisfactory churn cleaning practices, high counts are not necessarily due to improper care of the churn. Plainly, unsatisfactory churn treatments, however, must result in unsatisfactory counts. This seems to be borne out in this study. For instance the **rinsing of the churn** with cold or lukewarm water after the first day's washing would not produce a dry churn, and a moist churn promotes mold growth. This practice was reported by plants No. 9, 22, 1, 16, and 3 and may therefore have been, at least in part, the cause of the less satisfactory results obtained by these plants.

Plant No. 9 did not indicate the **amount of water** used and it had a count of 100 or less yeasts and molds per cc. in only 20 per cent of its entries and no counts below 50. Plants Nos. 20 and 16 did not indicate the amount of water used either. If too little water is used for washing so as to fill the churn less than $\frac{1}{3}$ full, though of a fairly high temperature, the water will cool quickly and the churn will not become hot enough to destroy the yeasts and molds nor dry well after washing. Plants Nos. 22, 1, and 18 were using from 50–70 gals. of water at temperatures of 180–190° F. in churns usually handling 800–1100 lbs. of butter. They were, no doubt, using insufficient amounts of water, and oftentimes the water is not as hot as it is estimated to be, not being tested with a thermometer.

In some cases the washwater is probably not kept in contact with the revolving churn for a sufficient **period of time.** Thus plants Nos. 22, 13, 20 and 16 rotate the churn for only 5 minutes with water at 150–180° F., while plants Nos. 9 and 3 do not indicate the time used for washing.

Plant operator No. 22 indicates that his churn is like new inside and that he keeps a **fan** blowing across the doors of the churn when not in use. This practice may be excellent for drying the churn. At the same time, however, yeasts and molds as well as dust may actually be blown into the churn by the fan. It will be noticed that the counts of plant No. 22 are not very satisfactory, which may, of course, be due also to other causes. However, as pointed out by Olson and Hammer (13), better results might no doubt have been obtained by leaving the churn doors open but in a position $\frac{2}{3}$ of the way up and covered with a screen and muslin.

Plant No. 5 uses **steam** for sterilizing the churn. This practice did not

give particularly good results and it may seriously open and crack the wood due to sudden expansion and later contraction. Blistering of the outside paint may be another objectionable result, and it does not provide the slushing action of hot water. Only plant No. 1 used a starter which may at times carry yeasts and molds into the butter. Thus, criticisms can be made with all of the methods reported by the plants with unsatisfactory yeast and mold counts except in the case of plant No. 12. If the details of the methods used by this plant were reported correctly, little fault could be found with them. However, the author knows from personal observation that the plant was seriously infected with mold, which may have caused the contamination from the air, although, of course, the high counts may also have been due to inefficient pasteurization and other causes.

Table 2 shows no direct correlation between the median yeast and mold counts of individual plants and the average flavor scores of their butter when fresh or when one month old. However, when the plants are grouped in accordance with their median yeast and mold counts, the average scores of the groups decrease as the counts increase.

The grouping of plants in table 2 is the same as that used in table 1. Since the loss in score during storage is usually higher with the high scoring butter than with low scoring butter, the samples of butter of each plant were separated into a high scoring and low scoring group, the former scoring over 35 in flavor and the latter 35 or less. The first group of plants with median yeast and mold counts of less than 50 per ml. had 138 samples of high scoring and 24 samples of low scoring butter, the second group with yeast and mold counts of 50–100 per ml. had 62 high scoring and 35 low scoring samples and the third group with the highest yeast and mold counts sent in 32 high scoring and 52 low scoring samples.

The average flavor scores when fresh of the three groups of plants were 37.10, 36.36 and 35.86 for the better butter and 34.66, 34.62 and 34.55 for the poorer butter samples. After storage for one month at 36–41° F. the average flavor scores for the three groups of plants and the two types of butter were 35.94, 35.21 and 34.49 and 34.41, 34.33 and 34.14 respectively indicating uniformly lower average scores when fresh and after storage for the plants with the higher yeast and mold counts.

The loss in score during storage also increases as the yeast and mold count increases. This increase in score losses is perhaps more significant than the figures indicate since usually the losses are smaller as the score of the fresh butter decreases. For that reason it seems that the high losses in score on the low scoring samples are a significant indication of reduced keeping quality in the case of the samples with high yeast and mold counts.

SUMMARY AND CONCLUSIONS

The methods regularly employed for washing the churns in seventeen different creameries in the State of Washington were studied in the light of

TABLE 2

Yeast and mold counts of butter as related to flavor score and keeping quality

NO. OF CREAMERY	MEDIAN YEAST AND MOLD COUNT PER ML.	BUTTER SAMPLES SCORING ABOVE 35 IN FLAVOR				BUTTER SAMPLES SCORING 35 OR LESS IN FLAVOR			
		No. of samples	Ave. flavor score when		Loss in score	No. of samples	Ave. flavor score when		Loss in score
			fresh	1 mo. old			fresh	1 mo. old	
23	17.5	28	37.05	35.78	1.27	0	.....	.....	.....
24	20.0	15	37.67	36.23	1.44	0	.....	.....	.....
11	22.5	10	35.70	34.55	1.15	10	34.56	34.22	0.34
14	30.0	28	37.19	36.06	1.13	0	.....	.....	.....
4	35.0	9	35.67	35.11	0.56	11	34.75	34.59	0.16
19	40.0	23	37.57	36.31	1.26	1	34.00	34.50	−0.50
21	40.0	25	37.38	36.29	1.09	2	35.00	34.25	0.75
	Below 50	138	37.10	35.94	1.16	24	34.66	34.41	0.25
5	72.5	16	36.05	34.77	1.28	4	34.83	34.00	0.83
3	80.0	11	35.77	34.91	0.86	9	34.67	34.14	0.53
13	90.0	18	37.47	36.13	1.34	0	.....	.....	.....
20	90.0	8	35.88	35.06	0.82	11	34.56	34.53	0.03
16	95.0	9	35.83	34.67	1.16	11	34.56	34.44	0.12
	50–100	62	36.36	35.21	1.15	35	34.62	34.33	0.29
1	110.0	8	35.75	34.81	0.94	9	34.50	34.28	0.22
18	215.0	6	35.92	34.58	1.34	16	34.50	33.93	0.57
22	287.5	12	35.93	34.43	1.50	4	34.63	34.75	−0.12
12	377.5	3	36.00	34.83	1.17	11	34.64	34.30	0.34
9	445.0	3	35.67	33.33	2.34	12	34.54	33.96	0.58
	100–500	32	35.86	34.49	1.37	52	34.55	34.14	0.41

the yeast and mold counts of the butter of these plants covering samples obtained during 14–28 different months.

Although there are many possible causes of high yeast and mold counts in butter, the consistently low counts of some of these plants indicate that their methods of churn treatment were satisfactory.

The methods of the seven plants having a consistently low yeast and mold content in their butter are given. From a study of these methods, the following factors appear to be important:

1. The use of ample quantities of washwater amounting to at least one-third to one-half the capacity of the churn.

2. A high temperature of the washwater, preferably 180°–200° F.

3. The use of 0.1–0.2 per cent of washing powder solution gives added protection by assisting in the removal of the grease, improving germicidal power and improving the odor of the churn.

4. Washing or rinsing with an alkaline crystalline hypochlorite solution of about 50 parts per million of chlorine together with hot water treatment also insured satisfactory results. Only four plants reported the use of this product, but three of these—Nos. 11, 4 and 19—were among the seven plants with the best yeast and mold records.

5. Keeping the hot water in contact with the revolving churn for at least 15 minutes.

Some fault could be found with the methods used by most of the other plants outside of the seven leaders, although, of course, the poor results obtained may have been due to other causes as well. Five plants mentioned rinsing the churn with cold or lukewarm water immediately after washing. Such a practice is undesirable because it does not leave the churn dry and a wet churn promotes microbial multiplication. In three plants too little water was used in washing. The small amount of water cools rapidly and thus loses its germicidal effect. In four plants the churns were in contact with the hot water for only 5 minutes, which is an insufficient period. One plant using steam sterilization did not produce satisfactory results.

Considering individual samples and the samples from individual plants, no direct relationship was found between yeast and mold counts and the quality and keeping quality of the butter held one month at 36–41° F. However, when grouping the plants in accordance with their median yeast and mold counts, the average scores of each group, both when fresh and after storage for a month at 36–41° F., decreased and the average score losses during storage increased as the median yeast and mold counts increased. Although the results do not prove any direct effect of yeast and mold content on the quality and keeping quality of commercial salted butter, the relationships found are of considerable significance.

It is apparent that in many of our commercial plants more attention might profitably be paid to a more painstaking care of the churn.

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DETERMINATION OF FAT, MOISTURE, AND SALT IN HARD CHEESE

BY THE SUBCOMMITTEE FOR THE ANALYSIS OF CHEESE
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The subcommittee on the analysis of cheese was appointed in 1933. The committee now submits to the members of the American Dairy Science Association on outline of the methods for the determination of fat, moisture, and salt in hard cheese. The committee expects a little later to submit another report dealing with the analysis of semi-hard and soft cheese.

DETERMINATION OF FAT, MOISTURE, AND SALT IN HARD CHEESE

1. *Sampling* (A.O.A.C. Method, slightly modified)

When the cheese can be cut, take a narrow, wedge-shaped segment reaching from the outer edge to the center of the cheese. Cut this into strips and pass three times through a sausage machine. When the cheese cannot be cut, take the sample with a cheese trier. If only one plug can be obtained, take it perpendicularly to the surface of the cheese at a point ½ the distance from the edge to the center and extending either entirely or half way through it. Where possible draw three plugs perpendicularly, one from the center, one from a point 1 inch from the outer edge, and one from a point half way between the other two. For inspection purposes reject ½ inch nearest the rind, but for investigations requiring the absolute quantity of fat in the cheese, include the rind with the sample. Grind the plugs in a sausage machine (the preferable method) or cut them very finely and mix thoroughly.

All samples unless analyzed immediately are wrapped in foil or similar non-absorbent material and are inclosed in small, air-tight, stoppered containers and analyzed as soon as possible without unnecessary exposure to the air. Samples not analyzed at once are kept in a refrigerator.

2. *Determination of Fat*

(1) Weigh 9 grams of cheese as prepared above into a dry, tared, 9 gram 50 per cent large-bodied Babcock test bottle. A specially constructed bottle having an opening in the upper part of the bulb and closed with a rubber stopper may be used. The rubber stopper should be discarded when it becomes hard. This operation should be done quickly so as to prevent, as much as possible, evaporation of moisture from the cheese.

(2) Add 12 cc. water at a temperature of 160° to 170° F. Mix well with the cheese.

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(3) Add in several installments 17.5 cc. sulphuric acid (sp. gr. 1.82 to 1.83), shaking the bottle after each addition of acid. Leave the bottle stand until all particles of cheese have dissolved.

(4) Centrifuge and add water as when testing cream.

(5) Place the bottle in a water bath 130° to 140° F., with the water level above the level of the fat. After 5 minutes add glymol and read the percentage of fat. Duplicate samples should check within 0.5 per cent.

3. *Determination of Moisture*

(a) For laboratories.

(1) The moisture dishes and covers are dried for 1 hour at 100° C. and allowed to cool for ½ hour in a desiccator containing sulphuric acid or other desiccant.

(2) A cover is placed on the dish and both are weighed on a chemical balance, preferably chainomatic.

(3) Approximately 2 to 3 grams of the sample are placed in the dish, the cover is immediately replaced, and a second weighing is made. Tests should always be made in duplicate or triplicate for the greatest accuracy.

(4) The samples are dried, with covers, in an oven at 100° C. If a vacuum of 20 inches or more is available, 10 hours' drying should suffice; if no vacuum is used, 24 hours' drying is recommended. Spattering may be minimized by placing the samples in the oven when the oven temperature is below 50° C. so that the samples are heated slowly. Covered dishes must be used to avoid losses of cheese when samples are placed in a hot oven. Vacuum should be applied slowly and released slowly.

(5) After drying, the samples are placed in a desiccator for about one hour, or until they reach room temperature, and each is weighed, without further delay.

(6) Loss in weight divided by weight of sample multiplied by 100 equals percentage of moisture.

Either 30 cc. pyrex beakers or aluminum dishes approximately 50 mm. in diameter and 22 mm. deep may be used. Each dish should be plainly and permanently numbered.

For routine laboratory analysis, if a balance having a tare beam and beams for direct readings and possessing a sensibility reciprocal* of 15 mg. is available, the above procedure is used with the following modifications:

(1) A dish 3 inches in diameter and 1 inch deep is used.

(2) Exactly 10 grams of the freshly-prepared sample are quickly weighed into the dish.

* The pointer should be deflected a distance equal to one division on the graduated portion when 15 mg. are placed on either scale pan when the scale is loaded to capacity.

(3) A cover is placed loosely on the dish. After being dried in an oven and cooled in a desiccator, the dish and cover are placed on the balance, and the percentage of moisture is read on the beams to the nearest 0.1 per cent. Duplicate samples when properly dried should check within 0.2 per cent.

(b) For cheese factories.

(1) Tare a dry, aluminum dish with cover on a balance which is equipped with a tare beam and with beams that permit the direct reading of the percentage of moisture. The balance should have a sensibility reciprocal of 15 mg. If the dish is first heated in order to dry it, cooling it afterwards to room temperature is important. A dish 3 inches in diameter and about 1 inch deep is satisfactory.

(2) From the freshly prepared sample weigh 10 grams cheese into the dish. This operation should be done quickly. The lid is placed loosely on the dish so as to permit the escape of moisture. The lid prevents the escape of fat and casein if spattering occurs.

(3) The dish is placed in an oven and heated slowly to a temperature of 220° to 230° F. This temperature should be maintained for 24 hours. An electrically heated oven equipped with a heat regulating device is satisfactory for this purpose. If electricity is not available, a pressure steam oven may be used. Since steam is usually not available over a period of 24 hours in the average cheese-factory, with a steam pressure in the jacket of the oven of from 40 to 50 pounds, and a temperature in the oven of 290° F., the drying can be completed in 4 to 6 hours. The temperature should be increased over a period of 1 hour to that desired. This procedure will avoid boiling over of the cheese. Electrically heated ovens can also be used for this short drying treatment.

(4) When the dish is removed from the oven the lid is placed tightly on the dish and the dish with moisture-free material is placed on a cool surface to cool to room temperature. The dish is then placed on the balance and the percentage of moisture read on the beams to the nearest 0.1 per cent after equilibrium has been reached. Duplicate samples should check within 0.2 per cent.

4. *Determination of Salt* (sodium chloride)

Weigh accurately approximately 3 grams of ground or chopped cheese

into a 300 cc. Erlenmeyer flask and add 10 cc. of 0.1711 *N* silver nitrate solution, or an amount more than sufficient to combine with all of the chlorine. Add 15 cc. of halogen-free, chemically pure nitric acid and 50 cc. of water and boil. As the mixture boils add approximately 15 cc. of saturated potassium permanganate solution in 5 cc. portions. Boil until all cheese particles are digested. Dilute the solution to about 100 cc., decant off the liquid into a beaker, and wash the precipitate by adding 100 cc. of water and decanting again. Add 3 cc. of a saturated solution of ferric ammonium sulfate as an indicator and titrate the excess silver nitrate with 0.1711 *N* potassium or ammonium sulfocyanate. Run a blank on the reagents used, following the same procedure, except to add sugar to destroy the excess of permanganate. The number of cc. of silver nitrate used minus the titration value divided by the weight of sample equals the percentage of sodium chloride in the sample. The reagents are made up by standardization against a salt solution containing 10 grams of chemically pure, dry sodium chloride per liter.

THE RATE OF CHANGE IN THE VITAMIN A CONTENT OF MILK*

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Although it is well known that the vitamin A value of milk and butterfat varies with the ration fed the cow, there is little specific information concerning the rapidity of change in the vitamin A content of milk following a change in the diet. Much of the information relating to this problem is derived from experiments designed primarily for some other purpose (1–6).

Since milk and butter owe their vitamin A value to both carotene and vitamin A, *per se*, determinations of the intensity of yellow color or carotene content, as well as the vitamin A activity, have been used by some investigators for ascertaining the rapidity of response to a change in the vitamin A value of the ration. Thus, Palmer and Eckles (7) found that the carotene value of milk fat responded very rapidly to a change in the carotene content of the ration. Similar observations were made by Doan (8) who observed that the maximum change in color took place in two weeks. However, Tucker and Pfau (9) observed that longer periods were necessary. Watson, Bishop and Drummond (10) stated that a maximum color value was secured in 29 days after a change in the character of the ration, but no readings were made at intervening dates. Bauman, Steenbock, Beeson and Rupel (11) showed, with readings taken at five-day intervals, that both vitamin A and carotene content of butter increased very rapidly. Moore (12) apparently obtained depletion in 13 days on a vitamin A poor ration, and repletion in 17 days. On the other hand, Fraps, Copeland and Treichler (6) state that "the effect of a feed cannot be judged by tests covering short periods of time."

There have been wide variations in the length of feeding experiments designed to study the effects of various feeds on the carotene and vitamin A content of milk fat secreted by dairy cows. A study of the literature shows that these experiments vary from 16 days to three months or longer. More exact information regarding the rate of change in the vitamin A content of milk resulting from changes in the ration would enable research workers to judiciously select feeding periods of sufficient duration to measure the major effects of the diet and at the same time make possible the elimination of unnecessarily long feeding periods. By using the shortest possible test period that can be conducted with safety, more tests could be made with

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the same cows during a single lactation period, feed could be conserved and more work accomplished in a given time.

EXPERIMENTAL

This experiment was planned to measure the rapidity of change in carotene and vitamin A value of butterfat when the cow was changed from a diet of high vitamin A potency to one of low vitamin A potency and *vice versa*. Good grade alfalfa hay was used as the chief source of vitamin A in the ration of high vitamin A activity and timothy hay in the ration of low vitamin A activity.

Two Guernsey cows in the fifth and sixth months of their second lactation were used in these feeding trials. In order to assure nutritive conditions favorable to the production of butterfat of high vitamin A value, the cows were placed on the alfalfa hay ration, consisting of alfalfa hay, limited amounts of corn silage and a grain mixture of white corn, ground oats and linseed oilmeal. At the end of thirty days, milk samples were collected from the cows. In order to measure the vitamin A activity of the milk fat, the cream was separated, churned into butter and the butter samples assayed for carotene and vitamin A.

TABLE 1

Showing the Rations Fed the Cows, the Dates on which Butter Samples were Secured and the Carotene Contents of the Butterfat

SAMPLE NO.	DATE TAKEN	RATION FED	NUMBER OF DAYS AFTER LAST CHANGE IN RATION	CAROTENE MICROGRAMS/ GRAM (BUTTERFAT)
Check	1-30-35	Alfalfa Hay, Corn Silage, Grain	30	7.4
T-1	2-1-30		1	7.4
T-2	2-2-35		2	7.2
T-3	2-3-35		3	6.9
T-4	2-4-35		4	6.8
T-6	2-6-35	Timothy Hay, Corn Silage, Grain	6	6.6
T-8	2-8-35		8	6.5
T-11	2-11-35		11	6.4
T-14	2-14-35		14	6.4
T-18	2-18-35		18	6.4
T-21	2-21-35		21	6.4
A-1	2-23-35		1	6.6
A-2	2-24-35		2	6.7
A-3	2-25-35		3	6.9
A-4	2-26-35		4	7.0
A-5	2-27-35	Alfalfa Hay, Corn Silage, Grain	5	7.1
A-7	3-1-35		7	7.3
A-10	3-4-35		10	7.4
A-13	3-7-35		13	7.4
A-17	3-11-35		17	7.4
A-21	3-14-35		21	7.4

The cows were then placed on the low vitamin A ration (timothy hay). Butter samples were churned from milk samples which were collected periodically as shown in Table 1. In order to follow closely the progressive changes in the samples as soon as they were available, portions of each sample were analyzed immediately for carotene. The samples were rendered at 50° C. and the fat separated from the curd and water by filtration. Five gram samples were dissolved in petroleum ether and made up to 100 ml. Using the method of Schertz (13), the samples were analyzed in a Bausch and Lomb Spectrophotometer, using a similar solution prepared with decolorized butterfat to compensate for the transmittancy of the fat. These values are given in Table 1.

From these data, it became apparent that the change in carotene was very rapid and had reached an equilibrium with the ration in about eleven days, and that little was to be gained by carrying the test beyond twenty-one days. At this point the cows were again placed on the alfalfa hay ration and the change in carotene content was followed for another twenty-one days. These results are also given in Table 1.

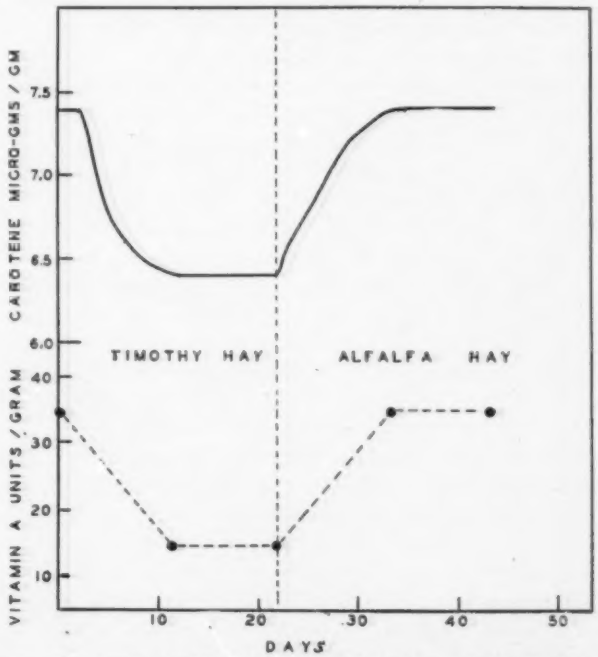

CHART 1. Showing the rapidity of response of carotene and vitamin A in the butterfat when the cows were changed from the high vitamin A diet to the low vitamin A diet and *vice versa*.

Biological assays for vitamin A were made on five selected butter samples. Since it was not feasible to assay all the samples taken, the five samples to be assayed were chosen at strategic points as indicated by the carotene content of the butters. The assumption was made that when the carotene had reached an equilibrium, the vitamin A activity had probably also reached an equilibrium with the ration. The vitamin A values of these samples were determined by biological assays, using the curative method as previously described (4). The results are given in Chart I, the values being expressed in Sherman and Munsell (14) vitamin A units.

DISCUSSION

From the results of these studies, as shown in Table 1 and Chart I, it becomes apparent that the carotene content of milk fat responds very rapidly to the carotene content of the ration fed the cow. It is interesting to note that when the cows were changed from a high carotene ration to a low carotene ration there was little change in the carotene content of milk fat produced within 24 hours, but beginning with the second day the carotene content declined very rapidly, apparently reaching a minimum for this ration at the end of eleven days.

When the alfalfa hay ration was substituted for the timothy hay ration, the carotene content of the butterfat produced rose immediately, being significantly higher at the end of the first day. The rise was rapid at first, then moderated until after the tenth day of alfalfa feeding when it seemed to be complete. No further change in the carotene content was observed on further feeding of the same ration.

Regarding the change in vitamin A taking place in the milk fat of these samples, it can be seen from Chart I that the decline in vitamin A content of the milk fat produced was virtually completed by the eleventh day after changing the cows from the alfalfa hay ration to the timothy hay ration. This is shown by the fact that ten days later (Sample T–21) the vitamin A content of the butterfat was almost identical with that of the butterfat produced on the eleventh day (Sample T–11). During the repletion period, since Samples A–10 and A–11 have almost identical vitamin A contents, it can be assumed that the increase in the vitamin A content of the butterfat produced was practically complete by the tenth day of alfalfa feeding.

In these experiments, it is apparent that there is a close correlation between the carotene content and the vitamin A value of the milk fat. With a decline in carotene there was a decline in vitamin A value and *vice versa*. However, this parallelism does not always exist. In other experiments (unpublished data) it has been found that the vitamin A value of butterfat may vary without materially changing the carotene content. Under certain conditions, especially those which affect the utilization of the vitamin of the ration or interfere with the vitamin A metabolism of the cow, it has

been observed that there may be little or no correlation between carotene content and vitamin A activity of butterfat.

The rapid response observed in these experiments is probably characteristic for the majority of feeding experiments, especially where the different rations contain some vitamin A activity. However, it must be recognized that when cows are placed on rations which are either totally deficient in vitamin A value of far below the bodily requirement of the cow, the initial decline in carotene and vitamin A will probably be similar to that observed in these experiments. After reaching the point where the carotene and vitamin A appearing in the butterfat are drawn from the tissue reserve of the animal, any further decline will be slow and gradual, depending on the rate of depletion of the body storage.

From the results of these feeding trials, it would seem that the transfer of the vitamin potency from the cow's ration to the milk is perhaps more rapid than many investigators have assumed. On the basis of these studies it would seem logical to conclude that the major effect of a feed upon the vitamin A content of milk might be measured in relatively short feeding periods.

This is not only significant from the standpoint of the research worker but should be of interest to the producer of milk and butterfat for human consumption. Dairymen attempting to produce milk or butter of high vitamin A value must of necessity watch the character of the rations fed his cows, avoiding feeding rations low in vitamin A potency ever for a short period of time.

SUMMARY

Studies were made of the rate of change in carotene and vitamin A content of milk fat from two cows following a change from a ration of high vitamin A value to one of low vitamin A value and *vice versa*. Alfalfa hay was the principal source of vitamin A in the high vitamin A ration while timothy hay was used as the principal source in the low vitamin A ration.

Following the change from a high vitamin A diet to a low vitamin A diet, the milk fat secreted by the cows declined very rapidly in carotene and vitamin A value, reaching an equilibrium level with the ration in about 11 days.

In the repletion period, the carotene content and vitamin A value of the milk fat increased very rapidly to an equilibrium level with the ration ten days after the cows were put on the high vitamin A ration (alfalfa).

It may be concluded that the major effect of a change in the diet upon the vitamin A content of cows' milk can be ascertained by relatively short feeding trials.

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THE COMPOSITION OF THE COLOSTRUM OF THE DAIRY GOAT

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The composition of the fluids and secretions of the mammary gland under normal and abnormal conditions is of interest due to the light that may be shed on the process of milk secretion and the part played by the various hormones in stimulating the initiation and maintenance of lactation. The amount of secretion which may be obtained from the smaller experimental animals in most cases is insufficient for analysis and difficult to remove. For many studies of milk secretion, the dairy goat is ideal because of its small size and large yield of milk. In conjunction with a study of the normal and experimental development of the mammary gland and of milk secretion in progress in our laboratory, it seemed highly desirable to determine the normal composition of the first secretion (colostrum) of the dairy goat for comparison later with the secretions which may be stimulated experimentally.

The composition of the colostrum of the goat has not been as widely studied as the colostrum of the cow. Henry in 1840 (1), (2) was the first to analyze the colostrum of the goat for a few major constituents including total solids, fat, casein, albumin and ash. Hucho (3), (4), Omneis (5) and Scheurlen (6) reported data on the specific gravity, total solids, fat, total protein, casein, albumin, lactose, and ash, but neither investigator included any data on the globulin content. Steinegger (7) studied the changes in colostrum of two goats for the morning and evening milk of the first day and observed further changes on the second, third and fifth days in one goat. Siegfeld (8), (9) analyzed the colostrum of two goats for a period of three consecutive days in the former study, and for four consecutive days in the latter study. He presented data for all the major constituents except albumin and globulin. Frahm (10) has presented the most complete data to date. He investigated the colostrum of three goats for a period of four, six, and seven days, respectively, and presented data for all the major constituents, but only partial data on the globulin content of the colostrum from one goat. Table I gives a summary of all available data from 1840 to date.

Practically all workers who have made analyses of milk, whether colostrum or normal have included non-protein nitrogen in their determination of total protein. As a result the total proteins have usually been too high.

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TABLE 1

Composition of the colostrum of the dairy goat (summary of literature)

NO. OF GOATS	NO. OF SAMPLES	SPECIFIC GRAVITY	WATER	TOTAL SOLIDS	FAT	TOTAL PROTEIN	CASEIN	ALBUMIN	GLOBULIN	LACTOSE	ASH	REMARKS
1	1		64.10	35.90	24.50	.....	5.20	3.20	.....	.....	3.00	Henry (1840)
1	1	1.0341	81.67	18.33	6.45	3.95	.....	.....	.....	5.69	0.87	Hucho (1897)
1	1	1.0321	83.38	11.62	2.80	.....	.....	.....	.....	2.60	0.71	Steinegger (1898) A.M. 1st day
	1	1.0371	87.90	12.10	3.30	.....		2.67	.....	2.40	0.58	Steinegger (1898) P.M. 1st day
	1	1.0314	87.21	12.79	3.90	.....		2.97	.....	2.54	0.61	Steinegger (1898) A.M. 2nd day
	1	1.0281	90.44	9.56	1.90	.....	.....	.....	.....	.....	0.64	Steinegger (1898) A.M. 3rd day
	1	1.0276	89.92	10.08	2.45	.....		3.19	.....	3.13	0.68	Steinegger (1898) A.M. 5th day
1	1	1.0276	89.95	10.05	6.10	.....		2.30	.....	3.15	0.78	Steinegger (1898) A.M. 3rd day
1	1	1.0538	77.23	22.77	5.20	.....		12.02	.....	2.84	1.00	Steinegger (1898) A.M. 1st day
	1	1.0360	.....	.....	16.85	.....		9.60	.....	2.24	0.99	Steinegger (1898) P.M. 1st day
	1	1.0300	87.20	12.80	4.20	.....	.....	.....	.....	.....	0.70	Steinegger (1898) P.M. 5th day
1	1	1.0643	70.80	29.20	6.45	.....	4.82	.....	.....	2.91	1.15	Omneis (1904)
1	1	1.0355	71.84	28.16	14.70	8.40	3.68	.....	.....	2.94	0.99	Siegfeld (1906) 1st day
	1	1.0330	84.50	15.50	5.10	4.14	2.16	.....	.....	4.45	0.84	Siegfeld (1906) 2nd day
	1	1.0330	84.46	15.54	5.50	4.46	2.28	.....	.....	4.42	0.88	Siegfeld (1906) 3rd day
1	1	1.0700	60.05	39.95	16.40	20.62	.....	.....	.....	3.50	1.27	Siegfeld (1908) 1st milking
	1	1.0348	82.60	17.40	6.85	.....	.....	.....	.....	.....	0.91	Siegfeld (1908) 2nd milking
	1	1.0333	82.07	17.93	7.40	.....	.....	.....	.....	4.57	0.90	Siegfeld (1908) 3rd milking
	1	1.0333	84.06	15.94	5.50	.....	.....	.....	.....	.....	0.90	Siegfeld (1908) 4th milking
1	1	1.0554	71.30	28.70	10.04	.....	4.88	4.33	.....	3.67	1.03	Scheurlen (1908) 1st day
3	5	1.0360	75.58	24.42	11.40	8.01	3.59	2.43	4.60(1)[1]	3.94	0.81	Frahm (1926) 1st day
3	7	1.0326	83.52	16.48	6.58	4.43	2.30	1.17	0.99(1)	4.52	0.86(5)	Frahm (1926) 2nd day
3	6	1.0316	84.55	15.45	5.36	4.27	2.61	0.95	0.48(2)	4.64	0.91	Frahm (1926) 3rd day
3	6	1.0338	84.78	12.22	5.35	4.13	2.61	0.87	0.46(1)	4.85	0.87	Frahm (1926) 4th day
2	4	1.0340	84.72	15.28	5.35	4.18	2.74	0.80	0.46(2)	4.69	0.77	Frahm (1926) 5th day
2	4	1.0343	85.60	14.40	4.52	3.98	2.56	0.81	0.46(2)	4.74	0.84	Frahm (1926) 6th day
1	2	1.0354	85.41	14.59	4.50	4.08	2.44	0.84	.....	4.84	0.88	Frahm (1926) 7th day
						Average of above data						
11	15	1.0429(14)	75.08(14)	24.92(14)	10.91		9.13(8)	4.06(9)	2.81(7)	4.60(1)	3.46(14)	1.04	1st day
6	10	1.0327	83.90	16.10	6.19		4.59(8)	3.53(8)	1.17(7)	0.99(1)	4.29(9)	0.83(8)	2nd day
7	10	1.0311	85.42	14.58	5.41		4.30(8)	2.56(7)	0.95(6)	0.48(2)	4.44(9)	0.87	3rd day
4	7	1.0337	84.66	15.34(6)	5.37(6)		4.13(6)	2.61(6)	0.87(5)	0.46(1)	4.85(6)	0.87	4th day
4	6	1.0323	86.00	14.00	4.68		4.18(4)	2.74(2)	0.80(4)	0.46(2)	4.68(5)	0.74	5th day
2	4	1.0343	85.60	14.40	4.52		3.98	2.56	0.81	0.46(2)	4.74	0.84	6th day
1	2	1.0354	85.41	14.59	4.50		4.08	2.44	0.84	.....	4.84	0.88	7th day

[1] The number in parenthesis indicates the number of samples used to arrive at the average figure.

Moir (11) called attention to this and suggested that the total protein be precipitated with warm trichloracetic acid, thereby leaving the non-protein nitrogen in solution. Likewise, casein has usually been precipitated with acetic acid at a pH of 4.2, which is considerably below its isoelectric point. Michaelis and Pechstein (12), by a series of cataphoresis experiments, found the isoelectric point of casein to be pH 4.6. Moir suggested the use of acetic acid, buffered with sodium acetate. This technique produces a filtrate with a pH from 4.5 to 4.7 and gave results with cow's milk which were usually from .1 to 2 per cent higher than those obtained by using acetic acid alone.

Due to the small proportion of globulin in milk, most investigators have been content to precipitate albumin and globulin together and report it as albumin. Moir developed a technique by which albumin and globulin may be determined separately. He suggested that the casein and globulin be precipitated with a saturated solution of magnesium sulfate. Then from total protein minus casein and globulin, albumin is obtained. Likewise, casein and globulin minus casein gives globulin.

Although the procedure for determining total protein, casein and globulin, and casein as used by Moir applied to normal cow's milk, we have, with slight modifications, found it adaptable for our work. We have studied the composition of colostrum for a period of nine days following parturition. The goats (grade Toggenberg) were milked twice daily and a composite sample taken of the morning and evening milk. Analyses were made on the first, second, third, fifth, seventh and ninth days.

METHODS OF ANALYSIS

Specific gravity was determined by means of a hydrometer at 15.6° C.; total solids by drying in an oven at 90 to 92° C. for 24 to 48 hours or until the sample ceased to lose weight; fat by the Babcock test; ash by drying the sample on a water bath and then ashing below redness. Lactose was determined by precipitating the proteins with zinc salts (13) and determining the amount of sugar present in the filtrate by using the Shaffer-Somogyi (14) copper-indometric blood sugar technique.

For the purpose of determining total protein, 5 ml. of a well mixed sample of milk were pipetted ino a weighed, covered beaker (50 to 100 ml.) and reweighed. Five ml. of 8 per cent trichloracetic acid were added to make the final concentration about 4 per cent. The mixture was maintained at a temperature of 60 to 65° C. on a water bath for 30 minutes, cooled, filtered, and washed several times with a 1 per cent solution of trichloracetic acid. The filter paper and precipitate were added to a Kjeldahl flask and the nitrogen content determined by the Kjeldahl method.

Casein and globulin were precipitated together by using a saturated solution of magnesium sulfate. Five ml. of milk (weighed as for total protein) were neutralized to phenolphthalein with N/10 sodium hydroxide, and

mixed with 45 to 50 ml. of a saturated magnesium sulphate solution. An additional amount of salt (anhydrous) was added to saturate 5 ml. of water. After standing for about an hour, the protein precipitate was filtered, washed with a saturated salt solution and its nitrogen content determined by the Kjeldahl method as for total protein.

Casein was precipitated at a pH of approximately 4.6 with acetic acid buffered by sodium acetate. Five ml. of milk (weighed as for total protein) were diluted with 25 ml. of distilled water warmed to 40 to 42° C. To this 0.75 ml. of 1.67 N (10 per cent) acetic acid was added at once and stirred gently by rotating the stirring rod in the beaker three or four times. After standing for 20 minutes, 2.3 ml. of 0.25 N sodium acetate solution were added. The filtrate was decanted and the precipitate washed with distilled water three times and then transferred to the filter paper, followed by one or two more washings. The nitrogen content was estimated as for total protein.

The albumin content of colostrum may be determined by subtracting the value for casein and globulin from the value for total protein. From the value for casein and globulin minus casein, globulin may be determined.

DISCUSSION OF THE DATA

The study of the composition of the first milk or colostrum and the gradual transition to normal milk is of considerable interest from several points of view. Not only does it indicate something of the rate at which the epithelial cells gradually take up the function of milk synthesis, but by means of the newer methods of protein analysis the transition in the globulin content of colostrum has been followed for the first time. The fact that immune bodies are transferred from the mother to the young in association with the globulin fraction of colostrum gives added importance to the separate analysis of this protein which is believed to pass from the blood stream into the milk unchanged (Woodman, 15).

At the same time the composition of goat's colostrum was of special interest in this laboratory as it is planned to try to stimulate lactation in the goat with the lactogenic hormone, galactin, and it is desirable to be able to compare the composition of the mammary secretions thus stimulated with the secretions stimulated by normal pregnancy (16).

The rapid transition of colostrum characterized by high total solids, fat and total protein into approximately normal milk is shown in Table II and Fig. 1, representing the average composition of the secretions of six Toggenburg goats during the nine days following parturition. An examination of these data indicate that a decided drop in several of the constituents occurred on the second day and that the milk tends to approach normality between the third and fourth days. The only constituent showing a slight increase during this period was lactose (Fig. 2).

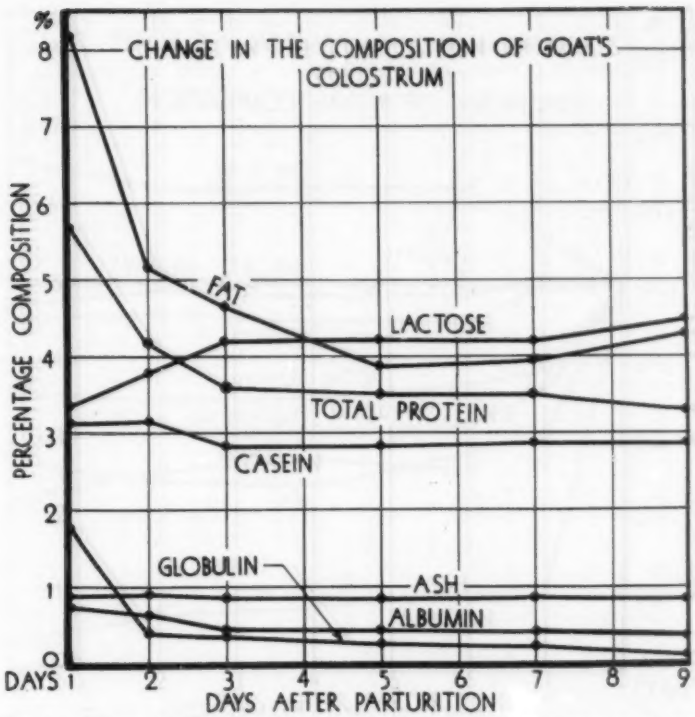

FIG. 1. Change in the composition of goats' colostrum. This average of the milk of six Toggenburg goats shows a rapid decline in fat and total protein (consisting chiefly of globulin). The lactose increases slightly and the ash decreases slightly as would be expected from their effect upon the osmotic equilibrium.

The fluctuations in the lactose and ash contents of colostrum are of interest due to the fact that these substances are chiefly responsible for the osmotic pressure of milk (Jackson and Rothera, 17). To maintain osmotic equilibrium alterations in lactose would require changes in the salts. An inverse relation between lactose and ash may be observed.

Total proteins are somewhat lower than those reported by other investigators. This is, in part, due to the fact that we have not included non-protein nitrogen in our results. On the other hand, the casein is higher than that reported by Frahm. As has been mentioned previously, Moir showed that by precipitating casein from cow's milk at the isoelectric point of pH 4.6 the casein content was increased 1 to 2 per cent. We have used the same procedure on goat colostrum and a slight increase may be attributed to this technique. A gradual decrease in albumin was observed during this period, being 0.78 and 0.34 per cent on the first and ninth day, respectively.

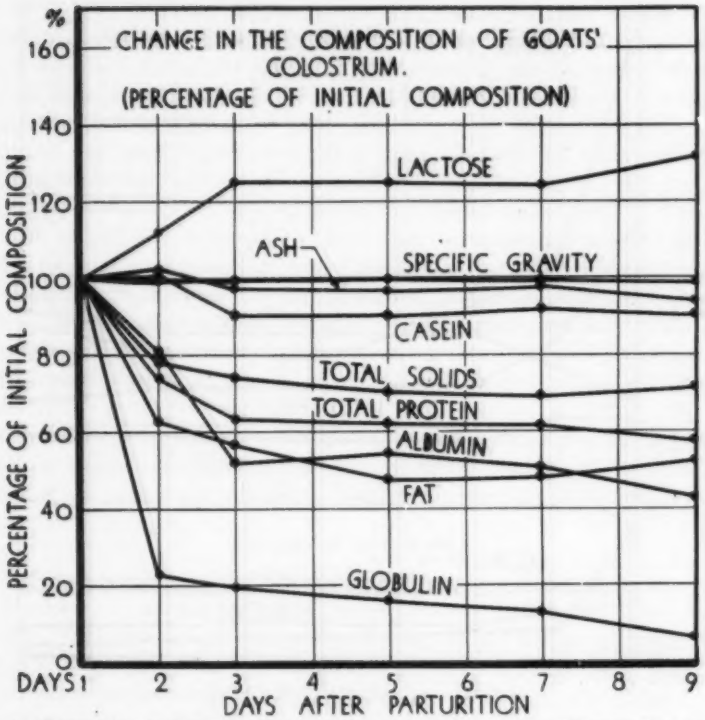

FIG. 2. Change in the composition of goats' colostrum based upon the percentage of the initial composition.

The most rapid change from colostrum to normal milk occurred in the globulin, which was 1.76 per cent on the first day, 0.40 per cent on the second day, then gradually declined to 0.11 per cent on the ninth day. Figures for comparison are few, since in most cases globulin and albumin have been reported together. It is of interest to note in the review of the literature that Frahm was the first to report partial data on one goat. He found the globulin to be 4.60 and 0.46 per cent on the first and sixth days. Because of the interest in the albumin and globulin fractions, the individual analyses are presented in Table III. In all cases the first milking was quite high in globulin with a marked drop at the second milking. This would indicate the importance of the kid receiving the first milk in order to obtain the largest amount of globulin and the associated immune bodies. The decline in the albumin content is quite gradual. These results indicate that in the goat, the large amount of total protein in the colostrum immediately after parturition is due to the globulin rather than the albumin.

TABLE II

The composition of goats' colostrum (avc. of six goats)

DAYS AFTER PARTURITION	SPECIFIC GRAVITY	WATER	TOTAL SOLIDS	FAT	TOTAL PROTEIN	CASEIN	CASEIN GLOBULIN	ALBUMIN	GLOBULIN	LACTOSE	ASH
1*	1.039	81.17	18.83	8.21	5.69	3.14	4.90	0.79	1.76	3.39	0.88
2	1.035	85.26	14.74	5.15	4.21	3.18	3.58	0.64	0.40	3.80	0.90
3	1.034	86.03	13.97	4.64	3.60	2.84	3.19	0.41	0.35	4.23	0.86
5	1.034	86.73	13.27	3.88	3.53	2.83	3.11	0.43	0.28	4.24	0.85
7	1.035	86.91	13.09	3.95	3.52	2.89	3.12	0.40	0.23	4.21	0.86
9	1.035	86.43	13.57	4.33	3.31	2.86	2.97	0.34	0.11	4.47	0.83

* In one doe the first milk was removed by the kid born during the night. This sample was eliminated from the average.

TABLE III

Albumin and globulin content of colostrum of individual goats

NO. DAYS	GOAT NUMBER												AVERAGE	
	83		66		43		254		57A		65			
	Albumin	Globulin	Albumin	Globulin	Albumin	Globulin	Albumin	Globulin	Albumin	Globulin	Albumin	Globulin	Albumin	Globulin
1	0.81	2.04	0.72*	0.37*	0.81	2.97	0.94	1.03	0.60	1.45	0.77	1.33	0.79	1.76
2	0.59	0.49	0.89	0.38	0.71	0.36	0.40	0.39	0.61	0.38	0.63	0.39	0.64	0.40
3	0.29	0.59	0.41	0.40	0.50	0.37	0.46	0.27	0.47	0.36	0.35	0.22	0.41	0.35
5	0.51	0.30	0.39	0.28	0.51	0.20	0.36	0.27	0.49	0.35	0.31	0.25	0.43	0.28
7	0.49	0.46	0.38	0.25	0.51	0.21	0.44	0.11	0.32	0.16	0.29	0.16	0.40	0.23
9	0.31	0.07	0.39	0.11	0.47	0.12	0.23	0.09	0.31	0.13	0.33	0.13	0.34	0.11

* The first milk was removed by the kid born during the night.

In comparing the transition of colostrum to normal milk in the goat with that in cattle (Sato, Ogura, Ikejima, 18) it would appear that most constituents show the same general trend. Fat, however, appears to be an exception being high in the goat immediately after parturition but generally low in the cow.

SUMMARY

1. The composition of colostrum and the transition to normal milk of six goats has been studied for a period of nine days. All the constituents decrease rapidly on the second day, with the exception of lactose which showed an increase.

2. Goat colostrum has a tendency to approach normality between the third and fourth days.

3. The newer methods of protein analysis have been used in a quantitative determination of these constituents present in goat colostrum. Total protein, free from non-protein nitrogen, was obtained by precipitating with 8 per cent trichloracetic acid. Casein was precipitated at its isoelectric point of pH 4.6 with an acetate buffer solution.

4. Albumin and globulin were determined separately. By salting out with $MgSO_4$, a quantitative estimation of the globulin content and its transition to normal has been followed for the first time. After the first milk is removed from the goat the globulin decreases very rapidly while only a moderate decline in albumin occurs.

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THE RELATION OF THE OXIDATION-REDUCTION POTENTIAL OF MILK TO OXIDIZED FLAVOR

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A survey of the literature on the problem of oxidized flavors in milk leads to the conclusion that there are at least seven or eight different factors which, operating alone or in combination, play a part in the production of this flavor.

Hammer and Cordes (6), Frazier (3) and others have shown that sunlight will cause a flavor in milk similar to that produced by copper and iron.

The variation in the tendency of milk to develop oxidized flavor as observed by Kende (9), Henderson and Roadhouse (7), and Prewitt and Parfitt (11) indicates that both the feed and the individuality of the cow are factors which govern the susceptibility of the milk to oxidized flavor development. It is postulated that certain feeds, particularly green feeds, impart an antioxidant to the milk, which accounts, in part, for the lessened tendency for oxidized flavor development in summer.

Henderson and Roadhouse (7), state that cows on submaintenance rations produce a more unsaturated fat which is more susceptible to oxidation.

Guthrie and Brueckner (5) emphasize the individuality of the cow as a factor. In an extensive study on milk from 155 cows of the Cornell herd drawn and pasteurized in brown glass bottles and protected from metals, they found that 21% of the cows gave milk which developed distinctly oxidized flavor when stored at 4.5° C. for three days. There was also a variation in the tendency for oxidized flavor development in milk from the different quarters of the udder of the same cow.

Heating milk to 75° C.–80° C. for one-half hour will reduce the tendency for the flavor to develop. Kende (8) and Gondos (4) express their belief that an oxidizing enzyme (oleinase) present in milk catalyzes the reaction and that the enzyme is activated by copper. Kende (8) showed that bacteria in large numbers will prevent oxidized flavor in milk. The rôle of bacteria in this respect is not sufficiently understood. Davis (2) suggests that the inhibitive action is due to the removal of oxygen by the growing bacteria and the production of a reducing potential in the milk.

The factors that various workers have shown to be involved in oxidized flavor development may be classified as follows:

1. Variation in the nature of fat itself, or of the constituent giving rise to the flavor.

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2. Factors tending to promote oxidation.
   (a) Catalysis by light. (b) Catalysis by oleinase. (c) Catalysis by metals.
3. Factors tending to prevent oxidation.
   (a) Antioxidants. (b) Bacteria.
4. Factors which act in an undetermined way, possibly influencing some of the above.
   (a) Individuality of the cow and of the quarter of the udder.
   (b) Season of the year.

The physico-chemical aspects of oxidized flavor in milk have not been sufficiently studied. Too little is known of the status of the physical equilibria in the production or prevention of oxidized flavor in milk. Only moderate attention has been given to the relation of the oxidation-reduction potential to oxidize flavor in milk. If the development of cardboard flavor in milk is an oxidation process it is reasonable to suppose that the oxidation-reduction potential plays a part more or less prominent.

Morris and Sommer (10) observed that cream having a high oxidizing intensity did not keep well. The addition of a reducing agent to cream of high copper content prevented oxidation for 5 months at $-18°$ C. to $-23°$ C. The data of Tracy, Ramsey and Ruehe (13) indicate a definite relationship between oxidized flavor and the oxidation-reduction potential of milk and cream containing added copper. Thurston (12) showed that the salts of copper and iron invariably raise the oxidation potential of milk but states that the oxidation-reduction potential is of no value in predicting the possible behavior of the milk as regards its tendency to become tallowy.

The work here reported throws additional light on the interpretation of the oxidation-reduction potential in relation to the keeping quality of milk and its products. The data, for the most part, were obtained over an extended period of time from studies on mixed milk received at a large milk plant. This milk, therefore, may be considered as average milk, all "individuality" factors arising from the cow and extraordinary feeding régimes being nullified in the mixing. The only important factors that might operate to vary the behavior of such milk toward oxidized flavor development are the seasonal variations in the milk itself and the extent of metallic contamination during processing. This work was done to determine the relative importance of these two factors and of the oxidation-reduction potential in the development of oxidized flavors.

MEASUREMENT OF OXIDATION REDUCTION POTENTIALS

Before the actual experiment was begun attention was directed to the construction of an electrode which would eliminate the disturbing effects of glass-to-platinum seals. Davis (2) and Tracey and Ramsey (14) have

pointed out that this is the source of much of the difficulty in securing good agreement between duplicate electrodes during oxidation-reduction potential measurements.

Electrodes are usually assembled by sealing platinum foil or wire into the end of a glass tube and making contact through mercury placed inside the tube. Frequently the glass seals developed minute cracks which retain cleaning solutions or permit mercury to seep through and come in contact with the exposed electrode. This results in false potentials, and the electrode must be rebuilt.

Details of the electrode construction used throughout this experiment are as follows: 5 cm. of No. 26 B & S gauge platinum wire are fused to the edge of a piece of bright platinum foil 7 mm. square and .08″ thick. A copper wire about 10 cm. in length is welded to the other end of the platinum wire. The electrode is then sealed into about 8 cm. of small glass tubing, making a glass-to-platinum seal as close as possible to the copper wire.

Two electrodes of this type held in a 3-hole rubber stopper are placed in the sample, contained in a 150 ml. wide mouth extraction flask. When the electrodes are properly adjusted the glass seal does not come in contact with test sample. The Agar-KCl bridge is passed through the stopper when measurements are being made. The electrodes are stored in chromic acid cleaning solution in flasks similar to those used for the test sample.

Before using, the electrodes are rinsed thoroughly with distilled water. No advantage was found in drying the electrodes after rinsing. Figure 1 shows both the electrode construction and the position of the electrodes in the sample.

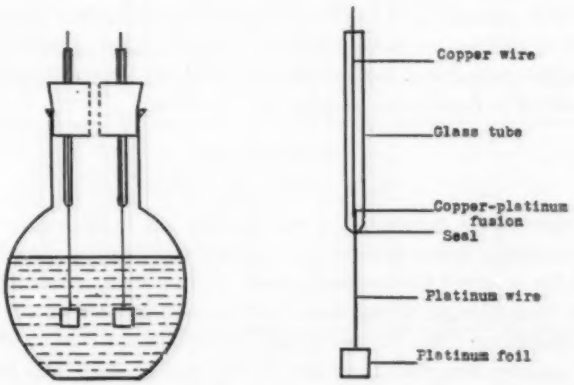

FIG. 1. The electrode construction and position in the sample.

Polarization of electrodes during measurements was avoided by using a vacuum tube circuit in conjunction with a Leeds-Northrup portable potentiometer. The circuit employed was essentially that described by Allyn and Baldwin (1). Minor modifications were made which enabled the instrument to operate on 22.5 instead of 90 volts.

To observe the oxidation potential trend of a milk sample, the electrodes were "aged" at least two hours in the sample before the initial reading was made. Without removing the electrodes after the first reading, the samples were stored at 4.5° C. and two more readings were made at 24-hour intervals thereafter. The three oxidation potential values so obtained indicated the potential trend of the milk. After the final reading was made, 50 hours from the time of bottling, the milk was tasted for evidence of oxidized flavor.

The theoretical correction of 0.06 volts for each unit pH difference between samples was not applied to the oxidation potential values because the pH variation would not be sufficient to affect the results.

Although the electrode design eliminated what was thought to be the chief cause of variation between duplicate electrodes, occasionally two identically treated electrodes were found which differed from 0.02 to 0.03 volts. When such was the case these electrodes were returned to the cleaning mixture and substitutions made. For the purpose of this investigation differences between duplicate electrodes of not more than 0.01 volt were considered satisfactory. However, in the majority of cases much better agreement was obtained. Not infrequently the electrodes checked within .0001 volt, or within the range of the galvanometer sensitivity. It will be apparent in the data that extreme accuracy of the oxidation-reduction potential values in a study of this nature is unnecessary. Even a slight agitation of the milk may alter the potential a few tenths of a millivolt. Nevertheless the values were recorded to four places as a matter of course.

EXPERIMENTAL

Study of Unmixed Milk

This part of the investigation was carried out to determine whether or not a relationship exists between the oxidation-reduction potential and the tendency for oxidized flavor development in milk from individual cows.

At the time this work was done Guthrie and Brueckner of Cornell (5) were investigating "The Cow as a Source of Oxidized Flavor of Milk." A series of quarter samples from cows of the college herd was furnished by these workers. The samples were selected to produce various degrees of oxidized flavor. The samples as received at the laboratory had been drawn and pasteurized in brown glass bottles and kept at 4.5° C. Although the first oxidation potential determinations on these samples were not made

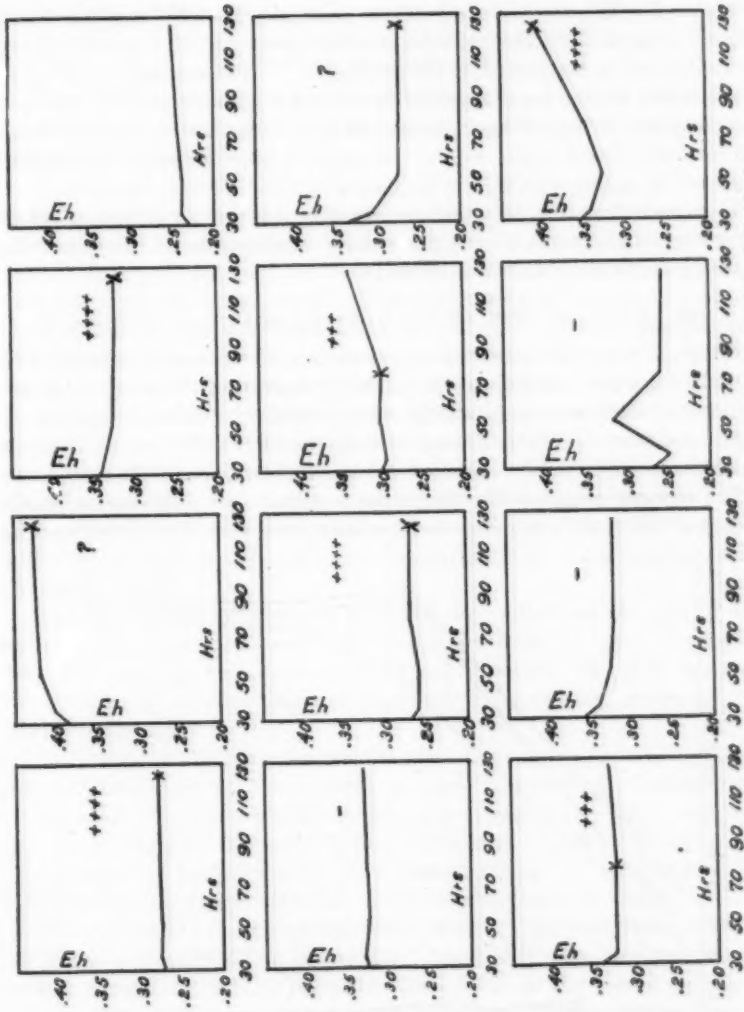

FIG. 2. Oxidation-reduction potential and flavor studies on milk from individual cows.

until 30 hours after milking, the first readings are comparable with the second readings on the milk used in a subsequent phase of this work.

The oxidation-reduction potential trend and the flavor results are shown in Figure 2. The points on the curves marked with "X" indicate where definite oxidized flavor development was first observed. The absence of any oxidized flavor is designated by the symbol "–." The number of "+" symbols indicates the degree of oxidized flavor which had developed in the samples at the end of the 125 hour storage period. A single "+" would indicate only a slight flavor and "++++" is used to show pronounced oxidized flavor.

It appears from the data that the absolute value of the oxidation-reduction potential of unmixed milk, pasteurized in glass, has no relation to the degree of oxidized flavor which develops.

THE EFFECT OF COPPER ON THE OXIDATION-REDUCTION POTENTIAL

Figure 3 shows the effect of copper sulfate added at the rates of 0.25, 0.50 and 1.0 p.p.m. copper on the oxidation-reduction potential of 50 per cent cream. Milk behaves similarly when treated in this way. The results confirm the work of Tracy, Ramsey and Ruehe (13) and Thurston (12) on the effect of copper on the oxidation-reduction potential of milk.

The increase in potential effected by copper is not proportional to the amount of the metal ion. This is especially true when the copper concen-

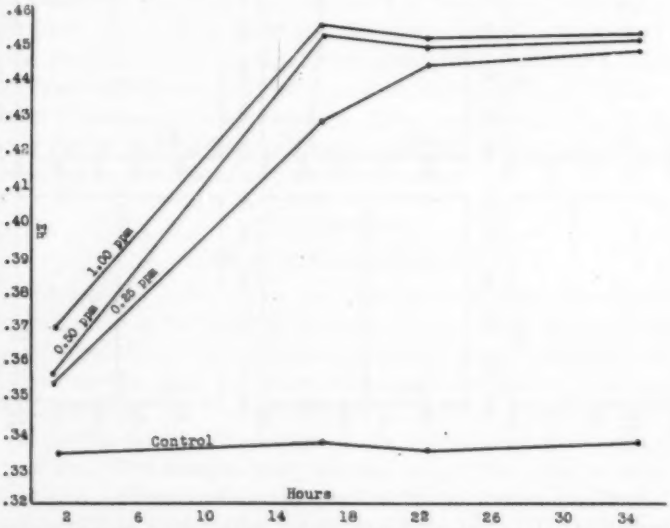

FIG. 3. The effect of copper on the oxidation-reduction potential of 50% cream stored at 4.5° C.

tration exceeds 0.5 p.p.m. The increase of over 0.1 Eh on the addition of 0.25 p.p.m. copper to the cream shows the extreme sensitivity of the oxidation-reduction potential system to amounts of copper comparable with those dissolved from plant equipment.

STUDIES ON MIXED MILK

The following data are the results of an experiment carried out at a large retail milk plant over a period of 16 months, beginning November 15, 1934, and ending March 20, 1936.

During the period from the beginning of this experiment to November 7, 1935, the hot pasteurized milk was pumped from the glass enamel pasteurizers to an internal tubular cooler. The conductor pipes from the pasteurizers carrying the hot milk to the cooler were made of tinned copper, as were also the tubes of the cooler. These pipes were old and worn and large areas of bare copper were exposed to the hot milk. During pasteurization the milk was in contact with a bare copper thermometer stem as well as a 5 foot length of 2″ bare copper pipe used as part of the discharge line.

During the winter the bottled milk of this plant consistently developed oxidized flavor. With the coming of the summer months the flavor became less pronounced, but would recur when pasture season was over. Twice a month, as regularly as possible, a sample of milk representing one lot was taken at three points in the system. These samples were: (1) Raw mixed milk from a large storage tank, (2) Pasteurized milk from the pasteurizer, (3) Bottled milk.

The first oxidation-reduction potential determinations on each of these samples were made not later than three hours from the time of sampling. Two more readings were taken after 24 and 48 hours. The milk was tasted for evidence of oxidized flavor immediately after the last potential determination was made.

In November, 1935, twelve months after the experiment was begun, the internal tubular cooler was replaced by a new tinned copper surface cooler. At the same time a gravity flow system was arranged for, which eliminated the bronze pump in the hot milk line. The worn conductor pipes were replaced, and the bare discharge pipes removed from the pasteurizers. All thermometer stems were retinned. Samples were taken as usual.

The experiment is divided into three periods. The first, from November 16, 1934, to May 14, 1935, covers the "dry feed" or winter period. The second, from June 4th to November 2nd, 1935, is the period during the greater part of which the cows were on green feed. It was during these two periods that the bare copper condition existed at the plant. The third period, from November 7, 1935, to March 20, 1936, is the second winter period. At the beginning of this period the bare copper was eliminated at the plant.

The oxidation potential and flavor data are shown in Figures 4, 5 and 6. The points plotted on the curves represent the average Eh of all the samples taken at the indicated place during a given period. The three readings, 24 hours apart, show the general trend of the oxidation-reduction potentials of the milk from the different points during the three periods.

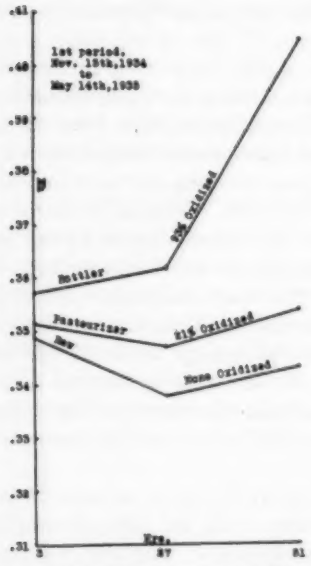

Fig. 4. Oxidation-Reduction Potentials And Flavor Of Milk During The Period From Nov.15,1934 to May 14,1935.

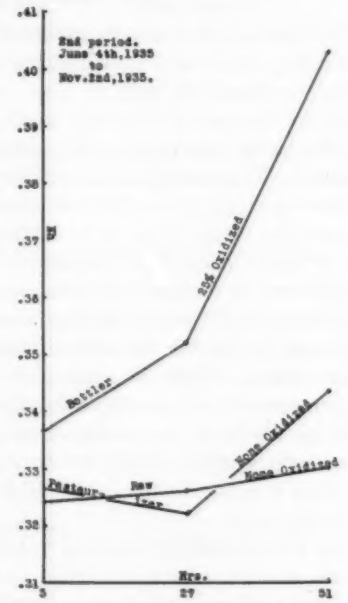

Fig. 5. Oxidation-Reduction Potentials and Flavor of Milk During The Period From June 4th, 1935 to November 2, 1935.

From Figure 4 it is evident that the flavor of mixed, processed winter milk is directly related to oxidation-reduction potentials. Of the samples from the pasteurizer, which had potentials slightly higher than the raw milk, 21 per cent developed oxidized flavor, while the much higher potentials in the samples from the bottler resulted in 93 per cent of these becoming oxidized.

The decreased susceptibility of the milk to oxidized flavor development in summer is shown in Figure 5. Of the samples from the raw supply and from the pasteurizer, none became oxidized. Only 25 per cent of the samples from the bottler became oxidized during the summer, despite the fact that the potentials were practically as high as during the winter.

Figure 6 shows the data for the third period. The installation of the new equipment at the beginning of this period, with elimination of the exposed copper, resulted in an immediate sharp drop in the potential of the

bottled milk, amounting to an average of 0.04 volt in the 48-hour-old milk. This drop in potential was accompanied by a corresponding decrease in oxidized flavor development, as compared with the previous winter. Only one sample, (14 per cent), of bottled milk became oxidized in this period, and this lot of milk was abnormal in that it showed the flavor in both raw and pasteurized samples as well as in the bottled milk. This was the only case of oxidized flavor in raw milk ever observed in this particular plant.

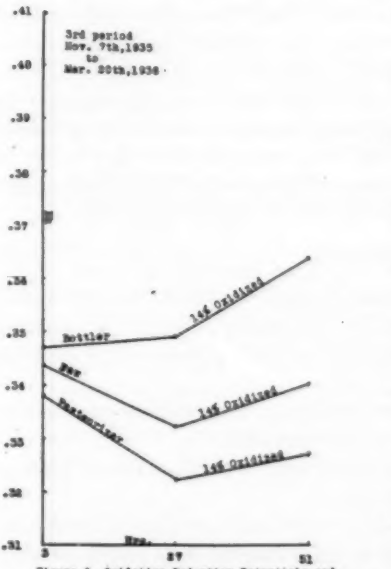

Figure 6. Oxidation-Reduction Potentials and Flavor of Milk During the Period From Nov. 7, 1935 to March 20, 1936.

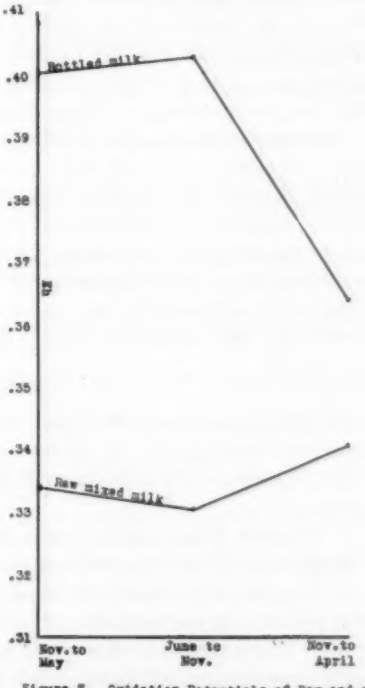

Figure 7. Oxidation Potentials of Raw and of Pasteurized Bottled Milk Over the Period November 1934 to April 1936.

In Figure 7 the average Eh of the 48-hour-old raw milk for the three periods is plotted. There is also plotted the average Eh of the 48-hour-old bottled milk. This shows clearly that the copper dissolved in the milk from the equipment throughout the first two periods caused the potential of the bottled milk to be high, while the potential was much lower during the third period, after the exposed copper had been eliminated.

The graphs presented here contain no data having any bearing on the effect of light or of the structure of the fat on oxidized flavor development. The oxidation-reduction potentials do offer a very reasonable explanation of

the effect of copper in catalyzing oxidized flavor development. Apparently copper produces the flavor in winter milk by raising the potential to a point sufficiently high so that some milk constituent is affected. In the summer season this same potential is not high enough to cause flavor changes. In these experiments bacteria could not have been responsible for the summer protection, since the bacteria count in the pasteurized milk was not high enough to keep the potential down, although it is usually thought that bacteria protect against flavor changes by producing a reducing potential. Whether the failure of the high potentials to bring about flavor changes during the summer was due to absence of oleinase, or to the presence of an antioxidant, or to a change in the structure of the milk constituent responsible, cannot be determined from these data.

The data on the samples of milk from the Cornell Herd show that oxidized flavor can occur in the milk from individual cows without any apparent relationship to oxidation-reduction potentials. Guthrie and Brueckner (5) were able to retard or prevent oxidized flavor development in similar samples by high-temperature pasteurization, which tends to support the theory of Kende (8) that oleinase is the catalyst. If this is the case, then the mechanism of oxidation by oleinase does not involve the oxidation-reduction potential, and is therefore quite different from the mechanism of copper catalysis.

CONCLUSIONS

1. The development of oxidized flavors in milk by the addition of copper is due to or accompanied by an increase of the oxidation-reduction potential of the milk to a point sufficiently high to bring about a change in some milk constituent.

2. Summer milk is able to resist the development of oxidized flavors even in the presence of a high oxidation-reduction potential.

3. The decreased susceptibility of summer milk to oxidized flavor development does not appear to be due to bacteria.

4. It seems probable that mixed milk from a large number of herds will rarely develop oxidized flavor unless it is contaminated with copper or some other agent that will raise the oxidation-reduction potential.

5. There is no relationship between oxidized flavors and oxidation-reduction potentials of the milk of individual cows. Oxidized flavors in such samples can develop when the oxidation-reduction potential is very low.

6. Since the evidence in the literature seems to indicate that oxidized flavor in milk from individual cows is due to oleinase, the data presented here indicate that the mechanism of oxidation catalysis by oleinase is entirely different from the mechanism of the catalysis by copper, since the former does not involve high oxidation-reduction potentials, while the latter does.

7. Oxidation-reduction potential measurements afford a very delicate means of determining the source of copper contamination in a milk plant.

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A NOTE ON FEEDING VITAMIN A AND D CONCENTRATE IN COD-LIVER OIL TO CALVES

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New York and Cornell University Agricultural Experiment Stations, Geneva and Ithaca, N. Y.

For many years the unthrifty condition of dairy calves from 3 to 10 months of age at the New York Experiment Station and on farms of many good dairymen has been often observed. This unthrifty condition is shown by roughness of hide and hair, lack of alertness, and a tendency to chew wood. Consequently recent advertising and some research stating that dairy calves need a vitamin A and D supplement was welcomed, and a feeding trial was inaugurated to establish the effect of fortified cod-liver oil on the growth and health of calves under conditions of feeding regularly used at the New York Agricultural Experiment Station at Geneva.

It has been shown by several investigators, as summarized by Morrison,[1] that "vitamins A and D are of great importance in raising dairy cattle, for they often suffer from deficiencies of these vitamins." Nevertheless, "when calves are fed the usual type of satisfactory ration, including plenty of good hay, there is no need to add a vitamin A or D supplement." It was thought that the Experiment Station Jerseys were fed a good ration yet the calves in common with many other young calves, were not in the most thrifty condition.

PLAN OF EXPERIMENT

For the purpose of providing an ample supply of good hay, racks were constructed in each calf pen and alfalfa hay of average quality was before the calves at all times. The hay was somewhat coarse and never a bright green yet little of it was all brown in color.

The calves were fed mother's milk for 2 days. For 2 months they were fed pasteurized milk and skimmilk mixed in equal parts at the dairy, the mixture containing about 2.5 per cent milk fat. This milk was produced by 24 Jerseys and 7 Holsteins fed alfalfa hay, corn silage, and an 18 per cent protein grain ration consisting of corn 400, oats 300, linseed oil meal 400, distillers' grains 300, bran 570, salt 10, and steamed bone-meal 20 pounds. From 2½ to 6 months of age pasteurized skimmilk was fed. All milk was fed twice daily in quantities determined by age and breed. The Jersey calves secured a maximum of 3 quarts per feed of milk-skimmilk and a maximum of 4 quarts of skimmilk per feed, the Holsteins receiving 50 per cent more. From 3

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[1] F. B. Morrison, Feeds and Feeding, 20th Edition, 1936.

months to 6 months of age the calves had access to water once daily, thereafter twice daily.

Grain feeding began at 2 weeks of age and after the calves were 3 months old the grain was raised to a maximum of 4 pounds per day as rapidly as the calves would consume it. This usually occurred at 6 to 7 months of age. The grain ration consisted of the 18 per cent dairy-cow feed mixed in equal parts by weight with coarsely ground oats.

Four of the calves received no cod-liver oil supplement, the other four receiving vitamin A and D concentrate in cod-liver oil, Nopco XX,* in the following quantities. To each feeding of milk one cc. was added for the first month and for the next five months 2.5 cc. were added. From one to six months of age each cod-liver oil calf received 5 cc. daily in the milk. In addition, these calves also ate grain to which 0.25 per cent of cod-liver oil concentrate had ben added or a maximum of 4.5 cc. from the grain, this maximum always being secured after conclusion of feeding skimmilk.

The two lots of calves, each lot consisting of three Jersey heifers and one Holstein heifer, were weighed weekly and their appearance carefully observed.

TABLE 1

Influence of cod-liver oil concentrate on rate of growth of dairy calves as measured by average weight in pounds

AGE IN WEEKS	WEIGHT OF CALVES		AGE IN WEEKS	WEIGHT OF CALVES	
	Regular feed group	Cod-liver oil group		Regular feed group	Cod-liver oil group
0	63.5	63.5	21	240.5	255.5
1	65.0	67.2	22	254.0	267.7
2	70.5	73.2	23	264.7	279.2
3	78.2	80.0	24	275.8	290.0
4	84.2	86.5	25	288.2	301.2
5	89.7	94.5	26	294.8	313.5
6	97.5	102.0	27	308.5	325.8
7	105.0	110.8	28	317.5	338.5
8	114.0	118.7	29	331.2	347.0
9	124.0	125.2	30	335.5	354.0
10	133.8	134.5	31	348.2	364.8
11	140.8	144.5	32	360.2	370.2
12	151.0	154.0	33	369.2	383.5
13	162.0	163.2	34	380.8	388.8
14	171.2	176.2	35	387.0	396.5
15	179.5	187.5	36	393.5	409.5
16	189.0	196.0	37	399.7	416.5
17	198.0	210.2	38	408.8	425.5
18	209.0	218.0	39	416.2	431.8
19	222.5	230.5	40	425.2	440.0
20	231.2	245.5			

* Nopco XX, vitamin A and D concentrate and cod-liver oil, as used in these experiments contained 2000 U.S.P. vitamin A units and 250 U.S.P. vitamin D units per gram.

RESULTS

The average weekly weights of the two lots of calves for 40 weeks are shown in Table 1. Fortunately, the average weights of the two lots were identical at birth. Thereafter the calves fed cod-liver oil weighed slightly more than the other calves and this difference persisted throughout the feeding trial. It is probable that no significance can be attached to these slight differences in weight.

Throughout the feeding trials several interested persons unsuccessfully tried to select the calves in each group. It was evident, however, that if there was a difference in the calves it was for the calves fed cod-liver oil concentrate to show a slightly smoother condition of skin and hair. Diarrhea or colds were not troublesome with any of the calves.

A very surprising and unexpected result was that all eight calves always appeared in excellent condition and never developed that unthrifty condition, as evidenced by rough hair, dull appearance, and the occasional chewing of wood which is so common in calves at 3 to 10 months of age. It is believed that the improved condition of the calves was due to the use of hay racks which greatly increased consumption of alfalfa hay, more than double that consumed when hay was fed twice daily on the floor of the pen.

CONCLUSIONS

When calves were fed rather liberal quantities of partially skimmed milk, skimmilk, all the good alfalfa hay they could consume, and a good grain ration, the feeding of vitamin A and D concentrate with cod-liver oil did not increase growth during a 40 week feeding trial. If there was any difference in general health it was in favor of the calves fed vitamin A and D supplement but such differences, if present, were slight.

The experiment indicated the necessity of good hay being available to the calves at all times to secure maximum growth and a thrifty condition of health even though skimmilk and grain were fed in liberal quantities.

American Dairy Science Association Announcements

COMMITTEES OF THE MANUFACTURING SECTION

Under the ruling made by this Section at the June, 1936, meetings of the American Dairy Science Association it becomes the duty of the retiring chairman to appoint the committees of the Manufacturing Section for the next ensuing year.

In accordance with his ruling the following committee appointments are respectfully submitted.

L. M. Thurston, Retiring Chairman, Manufacturing Section.

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WORLD'S DAIRY CONGRESS

The eleventh World's Dairy Congress will be held in Berlin from August 22 to 29, 1937, according to announcements from the executive offices of the Congress. On November 1 a total of 387 scientific papers had been submitted for reading before the Congress. The scientific papers are grouped into four main divisions, namely, Section 1, Milk Production and Tropical Dairying; Section 2, Milk Processing and Treatment; Section 3, Legislation, Marketing, and Education; and Section 4, Dairy Machinery, Buildings, and Transportation.

There will be an International Dairy Exposition in Berlin during the week of the Congress. The exhibits will include all kinds of dairy equipment and apparatus. An International Products Contest will be held in three major groups of products, butter, cheese, and condensed and dried milk products.

Those persons who wish information about the World's Dairy Congress, about presenting papers, about the products contest, or about any aspect of the Congress should correspond with the General Secretary, W. Clauss, World's Dairy Congress, Lindenstrasse 28, Berlin S W 68, Germany.

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ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

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SPECIAL PUBLICATIONS

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ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

BACTERIOLOGY

1. The Vitamin Requirements of the Lactic Acid Bacteria. S. Orla-Jensen, N. C. Otte and A. Snog-Kjaer, Tech. College, Kopenhagen, Zentr. Bact. II, *94*, p. 434, 1936.

True lactic bacteria do not grow in milk deprived of the vitamins of the B-group. Besides the lactoflavin they need another thermostable substance which is alkali fast and influences also favorably the growth of yeast. This activator must be considered identical with pantothenic acid, the main constituent of bios. This means that vitamin B_2 must consist of bios and lactoflavin. The rod shaped lactic acid bacteria need more of both activators than the streptococci, especially more lactoflavin (0.5 mg per liter), they need also a third activator which is not adsorbed by charcoal and may be obtained after evaporation of whey with the first fraction of the lactose crystals. It is possible to determine the bios and lactoflavin content of various materials by adding them to carbon treated milk and titrating the acid after this milk has been incubated with a suitable lactic acid bacterium. K.J.D.

2. The Vitamin Requirements of Various Bacteria Except the Lactic Acid Bacteria in Milk. S. Orla-Jensen, N. C. Otte and A. Snog-Kjaer, Tech. College, Kopenhagen. Zentr. Bact. II, *94*, p. 447, 1936.

The propionic acid bacteria and the tetracocci need bios and lactoflavin; the latter do not form pigment in carbon treated milk (except *Sarcina flava*). The pseudolactic bacteria, the coli-aerogenes group, do not depend upon these activators to grow in pure synthetic media. The same is true with *Microbacterium lacticum*, whereas the hay- and potato-bacteria thrive even better without these activators. The fluorescent bacteria and *Bacterium pyocyaneum* on the contrary grow better in untreated milk. They also produce more pigment then, whereas *Bacterium prodigiosum* behaves the opposite way. It looks as if the bios is the substance which prevents this bacterium from producing pigment in milk. This would mean that prodigiosin acts like bios. If the bacterium is given enough bios in the medium, it does not need to build it by itself. Similar experience has been made with *Bacterium violaceum*. Chemically the fluorescein has been proven to be a flavine. K.J.D.

3. On Growth Factors Present in Peptones. S. Orla-Jensen, N. C. Otte and A. Snog-Kjaer, Tech. College, Kopenhagen. Zentr. Bact. II, *94*, p. 452, 1936.

Most commercial peptones used for bacteriological purposes have enough lactoflavin but their bios content, however, is only sufficient for the streptococci. The commercial proteolytic enzymes contain plenty of lactoflavin and bios and possess moreover some other activators which promote especially the growth of the intestinal lactic acid bacteria. K.J.D.

4. The Nitrogen Requirements of the Lactic Acid Bacteria. S. ORLA-JENSEN, N. C. OTTE AND SNOG-KJAER, Tech. College, Kopenhagen. Zentr. Bact. II, *94*, p. 460, 1936.

The true lactic acid bacteria are especially particular in their nitrogen requirements. The problem was to prove whether the lactic acid bacteria could feed on simple nitrogen sources if the necessary activators were given.

The authors reached the following conclusions: Whey made free from protein by acidifying contains all activators necessary for the development of lactic bacteria. On the other hand it is a very poor nitrogen source which makes it very suitable for investigations of nitrogen compounds necessary for lactic acid bacteria. Naturally the data need confirmation by additional experiments with synthetic media. Colloidal caseinates are as good a source of nitrogen as the peptones, although lactic acid bacteria occurring in milk do not excrete caseolytic enzymes. They grow, therefore, much better in milk than in whey for lactalbumen seems to have no importance at all for the nutrition of the lactic acid bacteria. The thermobacteria as well as the other lactic acid bacteria do not require tryptophane but they need more or less cysteine, tyrosine (or phenylalanine), lysine, histidine, arginine, glutamic acid, asparagine, and creatine. Glutathion may be harmful in some cases. The *streptobacteria* can utilize ammonium salts and traces of *cysteine*. Creatine, diketopiperazine and probably also glutamic acid may be useful. *Streptobacterium plantarum* likes histidine and lysine, *Streptobacterium casei*, however, does not. The streptococci can live on ammonium salts as the only nitrogen source, but prefer the presence of histidine and leucine. These experiments with synthetic amino acid media confirmed the results of the first part of these papers concerning the need of lactoflavin and bios for the development of lactic acid bacteria. It could be further shown that the rod forms probably require a third activator which can be obtained with the first fraction of the lactose crystals, if the whey is evaporated.

K.J.D.

5. The Metabolism of Single Cells of Different Strains of Streptococcus Lactis. J. SUPINSKA AND T. MATUSZEWSKI, Inst. of Microbiology and Agr. Industry, Varsovie. Zentr. Bact. II, *94*, p. 499, 1936.

It was shown that the cells of *Streptococcus lactis* ceased to be in the logarithmic phase of growth and are already in the so called initial stationary phase when growing in milk at 0.3 to 0.8 per cent lactic acid. Statistical analysis of the possible relationships between the volumes of the cells and

their vital functions showed that, on the average, the larger the cells the greater the acid production and that the rate of reproduction also proved to be directly proportional to these two vital functions. The calculation of the partial correlation coefficient, under elimination of the fermenting ability, revealed a distinct negative correlation between the volume of the cells and the rate of reproduction. K.J.D.

6. Differentiation of Lactic Acid Bacteria in Starters. W. C. SMIT, Tech. Hochsch. Delft, Holland. Zentr. Bact. II, *94*, p. 289, 1936.

For those doing control work in creameries it is of great interest to know if the starter contains enough aroma bacteria. It is impossible, however, to differentiate the lactic acid streptococci from the betacocci by means of the microscope or of any one of the common culture media. The author studied a series of special media and found that the milk powder agar of Ayers and Mudge (Formula A) was indeed suitable to distinguish *Streptococcus lactis* colonies from the betacocci colonies. The colonies of the former are surrounded by a turbid halo whereas the latter ones do not change the medium. The results are better, if instead of commercial peptone Orla-Jensen's casein peptone is used and if the agar concentration is lessened. The pH should not be allowed to exceed 7.0 before sterilization and the milk powder agar should always be used in a freshly prepared state (not older than 3–4 weeks). The plates should be incubated a few days at 25° C. K.J.D.

7. Contribution to the Knowledge of Alkali Formers in Milk. W. STORCK, Milchw. Forschungsanstalt in Kiel. Zentr. Bact. II, *94*, p. 295, 1936.

The prevalence of the nonacid formers in milk during the colder months permits the alkali formers to play an important rôle. Their identification and isolation is easy since there is no need of using enrichment media. Their colonies are readily recognized on chinablue-lactose agar by a distinct colorless halo in the blue-grey agar. The isolated alkali formers belonged to the following groups (in diminishing frequency): Micrococci, Corynebacteria, Mycobacteria, spore formers, streptococci, Alcaligenes, Fluorescens, Proteus and sarcina.

Their growth in milk and dairy products is generally detrimental to quality. These may cause sweet curdling slime production and off flavors in milk. Their fat splitting properties produces deterioration of butter. On the other hand many of them may be of some value for soft cheese making, since they are able to attack the casein. This ability is promoted by their salt tolerance and growth at cheese ripening temperatures. They do not survive pasteurizing temperatures. K.J.D.

8. Incidence and Significance of Beta Hemolytic Streptococci in Cultures From a Selected Group of Milk Handlers. FRANKLIN M. FOOTE, Yale Univ. School of Med., New Haven, Conn., and HENRY WELCH, D. EVELYN WEST AND EARLE K. BORMAN, Conn. State Dept. Health, Hartford, Conn. Am. J. of Pub. Health *26*, 799, August 1936.

The authors studied 756 throat and 756 nose cultures obtained weekly over a week period from 85 milk handlers employed at 5 dairies including distributors of raw and of pasteurized milk. Transportation of throat and nose swabbings through the mails resulted in a reduction of approximately 50 per cent in positive findings. Twenty of the 85 milk handlers harbored beta hemolytic streptococci at least once. Five of the 20 individuals were persistent carriers.

Results indicated that strains of beta hemolytic streptococci found in individuals in an average state of health are indistinguishable from strains of known pathogenicity for man and are potential human pathogens assuming the lytic action of any strain on human fibrin to be associated with its invasive power.

With regard to the problem of milk-borne streptococcus infections the results indicated that:

(a) Physical examinations alone are not sufficient for the detection of all carriers of beta hemolytic streptococci.

(b) Routine laboratory cultures are inadequate for the detection of all carriers, unless made more frequently than is practicable under ordinary administrative conditions.

(c) Beta hemolytic streptococci do not withstand drying and other factors associated with delay in transportation to the laboratory sufficiently well for the detection of carriers in a central laboratory with any adequate degree of completeness.

(d) Two types of carriers of these organisms, transient (or occasional) and persistent occur among milk handlers in an average state of health, and the organisms of this type are potentially pathogenic for man, should sufficient numbers find access to the milk.

(e) The percentage of persons harboring these organisms is too large to permit adequate control of milk-borne streptococcus infections by employing any practical measures to eliminate the carriers.

(f) Since the frequency of the carrier state seems to bear no close relationship to the frequency of milk-borne streptococcus infections, outbreaks probably occur only when a comparatively large inoculum of infecting organisms reaches the milk from an udder infected by a milker or, probably less often, from some other source.

(g) Consumers must be protected against milk-borne streptococcus infections by other means than by periodic physical and laboratory examinations. M.W.Y.

9. Practical Methods of Testing For Mastitis. J. M. ROSELL, Ministry of Agriculture of Quebec, Canada. Am. J. of Pub. Health *26*, p. 872, Sept., 1936.

Methods generally used for detecting mastitis are summarized. For rapid and efficient work, the author recommends that in testing a herd for mastitis, the order of tests to be used with quarter samples should be as follows: (a) pH with brom thymol blue; (b) rapid catalase with 9 per cent hydrogen peroxide; (c) rapid chloride test, and after or before this test, (d) the black sieve cloth test, and (e) palpation and clinical examination of the udder. M.W.Y.

10. An Outbreak of Epidemic Sore-Throat of Milk-Borne Origin. F. E. CAMPS AND J. L. MILLER. Lancet *231*, p. 756, Sept. 26, 1936.

An infected raw milk supply caused 1600 cases of sore-throat, mostly in adults, in and around Chelmsford in August, 1935. Many of these were reported as scarlet fever, although rashes occurred in only a limited number of cases. The source of the epidemic, in which 6 deaths occurred, was a farm where four cow men were found to have been infected with *Streptococcus pyogenes* (Type 2). This milk supply was shut off until it was pasteurized. The outbreak was a typical milk-borne one, with sudden outburst of disease quickly reaching a peak, and there were relatively few secondary cases. The schools were not closed. J.A.T.

11. Enteric Fever in Milk-Borne and Water-Borne Epidemics. A Comparison of Age- and Sex-Incidence. A. BRADFORD HILL AND K. MITRA. Lancet *231*, p. 589, Sept. 5, 1936.

In order to determine the truth of the hypothesis that a milk-borne epidemic of typhoid differs from a water-borne epidemic of the disease in the age and sex distributions of the persons attacked, the authors studied by statistical methods 28 epidemics due to water and 31 due to infected milk, which have occurred in Great Britain and North America.

In the early years of childhood there is no difference in the ratio of male to female patients between the water-borne and milk-borne epidemics. In the later ages of childhood, 5–14 years, there is no change in this ratio in the water-borne group as compared to the ratio at ages 0–4, but in epidemics attributed to milk the proportion of females becomes higher. In the adult years, over 20, the number of male and female patients in water-borne typhoid remains equal, but there is, on the average, a distinct excess of females among the patients of milk-borne typhoid.

In an editorial comment on this study, in the Lancet for September 12, 1936 (page 639), it is suggested, therefore, that a heavier incidence of typhoid cases during an epidemic upon children and, among adults, on females justifies some presumption that milk is at fault. This article, says the editorial, proves that statistics are neither dull nor unintelligible.

J.A.T.

BUTTER

12. Manufacturing Butter of the "Danish" Type. J. M. ROSELL, Provincial Dairy School, St. Hyacinth, P. G. Can. Dairy and Ice Cream J. *15*, pp. 18 and 61, Pan. and Feb. 1936.

An interesting discussion of the methods for making the "Danish" type or ripened cream butter for the British market is given. J.C.H.

13. Casein-Formalin Treatment of Butter Boxes. E. G. HOOD, Div. of Dairy Res., Ottawa. Can. Dairy and Ice Cream J. *15*, 7, p. 47, 1936.

The Casein-Formalin treatment of butter boxes is recommended for overcoming wood taint in storage butter. The author describes the equipment for spraying and gives formulas for spray material and their preparation and use. J.C.H.

14. Butter Wraps and Loss of Weight in Prints. A. H. WHITE, Div. of Dairy Res., Ottawa. Can. Dairy and Ice Cream J. *15*, 8 p. 24, 1936.

A comparison was made of the loss of weight of print butter in storage when wet and dry parchment, "M. A. T. cellophane," and "Pliofilm," manufactured from crude rubber, was used as wrappers. The weight loss was least for Pliofilm wrappers with slightly the greatest loss for parchment wrappers but no significant differences were found in the weight losses of print butter wrapped in the different wrappers.

No noticeable differences were observed in the surface flavors or the color of the prints in the different wrappers.

At the present time both "M. A. T. cellophane" and "Pliofilm" are more expensive than parchment paper which would be the determining factor for their use for print butter. J.C.H.

Other abstracts of interest are numbers 1, 4, 6, 26, 31, 32, and 38.

CHEESE

15. On the Behavior of a Few Pencillium-Species, Important in Dairy Technology, Towards Different Organic Nitrogen Sources. KARL J. DEMETER AND RICHARD PFUNDT, South German Res. Inst. for Dairying in Weihenstephan, Tech. College, Munich. Zentr. Bact. II, *95*, p. 54, 1936.

Some practical experiences led to the belief that the growth of *Pencillium bruneo-violaceum*, a mold very detrimental to camembert cheese, would be promoted in cheeses made from pasteurized milk. This might be explained by a possible preference for such nitrogen derived from the albumen content of cheeses made from pasteurized milk. A further practical experience was that cheeses which were oversalted were especially prone to the infection with this mold.

The aim of the authors was to clear up this question by a comparative

investigation on the organic nitrogen requirements of the following molds: *candidum, P. camemberti* and *P. bruneo-violaceum*. The organic nitrogen sources were the following: glykokoll, alanine, leucine, glutamic acid, cystine, peptone, albumen and casein. It was shown that albumen and its derivatives were not good nitrogen sources for *P. bruneo-violaceum* and that this mold was stimulated by peptone and glutamic acid especially if there was no alkaline reaction. This agreed with the practical experience that *P. bruneo-violaceum* thrives on very young cheeses. The destruction of casein was not uniform with the two strains used.

Concentrations of 5–15 per cent of sodium chloride are more detrimental to the true camembert molds than to the *P. bruneo-vioviolaceum*. This confirms the above mentioned practical experience concerning the effect of oversalting. Finally it may be mentioned, that in confirmation of the work of Moser, volutine never could be detected in cultures of *P. candidum*, always with *P. camemberti* and usually also with *P. bruneo-violaceum*. This fact might be used to differentiate between the two species of true camembert molds. K.J.D.

16. Butyric and Lactic Acid Fermentation in Silage Fodder. J. VAN BEYNUM AND J. W. PETTE, Agr. Exp. Station, Hoorn, Holland. Zentr. Bact. II, *94*, p. 413, 1936.

It was shown that in silage made with the addition of inorganic acids, butyric acid fermentation may take place, even if the average pH was less than 3.0 because the different layers with the acid solution were very unequal. The butyric acid fermentation could not take place in a silage of pH 3.0. From pH 3.5 upwards a lactic acid fermentation and a certain type of butyric acid bacilli, *Clostridium tyrobutyricum* always developed previous to the butyric acid fermentation. Therefore a silage in which a lactic and butyric acid fermentation has taken place is likely to cause contamination of milk to producing swelling of cheese.

The authors have further shown that a silage prepared according to the Dutch method always contains butyric acid and that it is always dangerous for cheese making, since in most cases a previous lactic acid fermentation has taken place. K.J.D.

Other abstracts of interest are numbers 1, 4, 6, 21, and 26.

CHEMISTRY

17. A Note on the Determination of Iodine in Biological Material. GLADYS J. FASHENA AND VIRGINIA TREVORROW, Dept. of Pediatrics, New York Hospital and Dept. of Biochemistry, Cornell Univ., Med. College, New York City. J. Biol. Chem. *114*, p. 351, 1936.

A discussion of factors which affect a micro-determination method for iodine in biological materials, and details of an improved technique.

K.G.W.

18. The Ionization Constants of Calcium Proteinate Determined by the Solubility of Calcium Carbonate. E. G. WEIR AND A. BAIRD HASTINGS, Dept. of Physiology and Lasker Foundation for Med. Research, Univ. of Chicago. J. Biol. Chem. *114*, p. 397, 1936.

The results of this study are consistent with the hypothesis that calcium combines with proteins to form salts which are partially ionized into CA^{++} and protein ions. Further, that an equilibrium exists between these ions and the un-ionized calcium protein salt, which may be expressed by the mass law equation. Determinations of the solubility of calcium carbonate in solutions of casein, serum albumin and serum globulin have been made at 38 degrees for different pH values and protein concentrates. The ionization constants of the calcium salts of these proteins have been calculated and found to have the following values: casein, $pK_{CaProt} = 2.23$; globulin, $pK_{CaProt} = 2.32$; albumin, $pK_{CaProt} = 2.20$. K.G.W.

19. Calculation of Isoelectric Zones and Isoeletric Points. DAVID M. HITCHCOCK, Lab. of Physiology Yale Univ., School of Med., New Haven, Conn. J. Biol. Chem. *114*, p. 373, 1936.

In this paper the author discusses the significance of curves of the dissociation of simple ampholytes with particular reference to the breadth of the isoelectric zone, the isoelectric points of multivalent ampholytes, and methods of expressing isoelectric points. K.G.W.

20. The Denaturation of Proteins by Sound Waves of Audible Frequencies. LESLIE A. CHAMBERS AND EARL W. FLOSDORF, Johnson Foundation for Med. Physics and Dept. of Bacteriology and Pediatrices, Univ. of Penn., Philadelphia, Pa. J. Biol. Chem. *114*. p. 75, 1936.

Intense sound waves when applied to certain protein solutions, will cause coagulation. It has been shown by investigation that sound-denatured albumin (protein of lower solubility than that of the native protein) is immunologically similar to denatured albumin prepared in other ways, *e.g.*, by heat in acid, alkaline, or neutral solution, by alcohol, or by acid or alkali. While previous studies indicate that the end-product of sonic denaturation is probably the same as that produced by heating and therefore may be the result of a thermal type of activation, they have not explained the mechanism through which mechanical vibrations may energize the reaction. The authors in this paper report a study of sonic denaturation of certain proteins, first, to compare the end products of the reactions with those produced by other means with respect to chemical properties, and second, to obtain evidence as to the nature of the energy transfer underlying the reactions. Two experimental sonic vibrating units were used in the experi-

ments, one having a resonant frequency of 1200 cycles per second, the other about 9000 cycles per second. With these instruments, egg albumin and plastein were denatured. The solubility of the products under various conditions of pH was the same as for the products denatured by heat. Horse serum albumin, one the other hand, was not denatured by sonic vibration which suggests that the mechanism of the sonic reaction is different from simple thermal activation. K.G.W.

Committee Comments: Sonic vibration is being studied experimentally to homogenize milk, cream, and ice cream.

CONCENTRATED AND DRY MILK

Abstracts of interest are numbers 21, 22, 23, 24, 25, 26, 27, 31, 32, 34, 35, 36 and 38.

FOOD VALUE

21. Lactoflavin, A Necessary Growth-Promoting Factor. S. ANSBACHER, G. C. SUPPLEE AND R. C. BENDER. The Dry Milk Company, Inc., Research Lab., Bainbridge, N. Y. J. Nutrition *11*, p. 401, May, 1936.

Lactoflavin is a necessary growth-promoting dietary factor. A difference in the rate of growth of white rats results from the difference in the daily intake level of pure crystalline lactoflavin varying from 2.0 to 20.0 gamma. The growth-promoting properties of lactoflavin are readily determined with a suitable basal ration adequately supplemented with pure vitamin B and a third factor or group of factors necessary for the prevention and cure of dermatitis and carried by rice polish. The potency of lactoflavin may be calculated to be 150,000 units per gram. However, it is emphasized that there is apparently a discrepancy between the potency of the pure lactoflavin and that contained in natural products. L.A.M.

22. The Iron and Copper Content of Milk Throughout the Season As Related to Anemia Development in Rats. W. E. KRAUSS AND R. G. WASHBURN, Dept. of Dairy Industry, Ohio Agr. Exp. Sta., Wooster, Ohio. J. Biol. Chem. *114*, p. 247, 1936.

Possible variations in the iron and copper content have been suggested as causes for certain nutritional and chemical properties of milk. In this report the authors investigated the iron and copper content of the milk of ten cows, seven Jerseys and three Holsteins, while on various types of rations, both winter and summer.

According to the methods of analysis used, the range in iron content for a period of about one year was 0.34 to 0.43 mg. per liter, and for copper, 0.14 to 0.19 mg. per liter; slightly greater variations were observed in individual, rather than the group analyses. The iron and copper intake when the cows were on dry feed was approximately three times that when they

had access to pasture. This wide range in intake was not reflected in the iron and copper content of the milk.

The rate of anemia development in pairs of rats fed samples of the milk (one pair receiving the milk raw, the other the milk pasteurized at 52.5° F. for 30 minutes) obtained during the course of the study indicate that the biological responses confirm the chemical data; there was not sufficient variation in iron and copper content from period to period to affect appreciably the rate of anemia development. The results demonstrate that no hematopolietic factor destroyed by pasteurization is imparted to milk when cows are under so-called ideal feeding conditions. K.G.W.

23. The Vitamin C Content of Human Milk and its Variation with Diet. Iva Selleg and C. G. King, Univ. of Pittsburgh, Pittsburgh, Pa. J. Nutrition *11*, p. 599, June, 1936.

The vitamin C content of human milk, as determined by the 2, 6-dichlorophenol-indophenol titration technic, was found to vary from 0.002 to 0.108 mg. per cubic centimeter. The average of fifty-three cases, 3 to 7 days postpartum, was 0.055 mg. per cubic centimeter. On a good hospital dietary without special supplements the average value rose gradually to 0.064 mg. on the tenth day. When the mother was receiving an adequate diet the usual range of vitamin C was in the zone of 0.060 to 0.080 mg. per cubic centimeter in milk. Cases with markedly subnormal antiscorbutic values rapidly approached normal when an orange juice supplement was given. The excess quantities of vitamin were eliminated rapidly in the urine. The investigation provides evidence against the suggestion that humans can synthesize adequate quantities of ascorbic acid during gestation. L.A.M.

24. Infantile Diarrhoea in Institutions. R. Carter. Lancet *231*, p. 162, July 18, 1936.

Malted milk in the treatment of enteritis in infants is recommended for its low fat, high carbohydrate value. After stopping all food for 24 hours, it is given in water every three hours in the proportion of 3 tablespoonsful of malted milk to 3 ounces of water. After about a week on the malted milk diet, fresh milk in small amounts or dried milk reliquefied in cream is added in gradually increasing quantities. By this means, the mortality from this disease has been reduced to only 4 per cent. J.A.T.

25. Prevention of Disease by Diet. A. G. Morrison, S. Datta and A. F. Waters. Lancet *230*, p. 1472, June 27, 1936.

The effect of improvement in diet on the incidence of dysentery and typhoid fever in an English institution is shown in this report.

An outbreak of dysentery in August 1932 resulted in 123 cases, in spite of every effort to isolate patients and prevent the spread of the disease. None of the staff was affected, and examination of milk and water supplies was negative.

A study of the patient's diet showed it to be deficient in protective foods, although the total calories and the first-class protein were more than adequate. Milk, fresh fruits and vegetables, fats, liver and eggs were inadequately provided.

The daily ration of milk was increased by at least 6 oz., and liver and fish were given at least once a week, while fresh fruits and vegetables were added daily. The margarine was vitaminized, and the meat ration was reduced.

Since September 1932 when the improved diet was inaugurated, there has been no case of dysentery and only three cases of enteric fever. From 1918 to 1932 there had been a fairly constant number of cases of typhoid fever, but there were none after 1932.

While these observations apply to only a limited group of patients for a comparatively short time, the authors believe that the results are significant of what may be accomplished when the diet is improved in general quality and vitamin A value in particular. J.A.T.

26. Relative Value of Raw and Heated Milk in Nutrition. E. C. V. Mattick and J. Golding. Lancet *231,* p. 702, Sept. 19, 1936.

In order to determine the relative food values of raw, pasteurized, and sterilized milks, the authors fed groups of rats on these milks and dry biscuits made of English flour. Five generations were raised on raw milk, three on pasteurized milk, and none on sterilized milk.

The results of this test and another, in which a comparison was made of rats fed on raw milk and on milk pasteurized at 145° to 150° F. (higher than the commercial usage), showed that there were no significant differences in the weights of the animals, nor in their retentions of calcium and phosphorus. Red cells and haemoglobin were also similar in both groups.

A loss of hair amongst rats of the second and third generation on pasteurized milk was, however, shown and is attributed by the authors to a possible deficiency in vitamin B.

Committee Comment: Competent investigators in the United States have raised more than 40 successive generations of healthy rats on diets of pasteurized and dried milk, and whole wheat, with no loss of hair. Apparently some factor is involved other than pasteurization. J.A.T.

27. The Influence of Dextrin and Sucrose on Growth and Dermatitis. R. C. Bender, S. Ansbacher, G. E. Flanigan and G. C. Supplee. The Dry Milk Company, Inc., Research Lab., Bainbridge, N. Y. J. Nutrition *11,* p. 391, May 1936.

A study has been made on the prevention of dermatitis in white rats fed a synthetic diet containing pure vitamin B and lactoflavin but no other part of the vitamin B-complex. Comparative data from basal rations containing dextrin and sucrose, respectively, show that no dermatitis resulted

when the former was used, whereas a high incidence of dermatitis resulted when sucrose served as the basal carbohydrate. Vitamin B and lactoflavin supplementing the sucrose ration did not prevent the development of dermatitis, nor did these supplements permit normal and continued growth. Such supplements fed with dextrin promoted a substantial rate of growth. A concentrate prepared from rice polish cured the dermatitis occurring in the sucrose fed animals and at the same time promoted a substantial growth, provided adequate amounts of vitamin B and lactoflavin were fed simultaneously. The occurrence of dermatitis was delayed and was not so regular with a sucrose ration containing 10 per cent hydrogenated vegetable oil as with one containing 3 per cent of the same oil. The data as a whole would seem to indicate that the basal ration containing 69 parts of sucrose and 3 parts of hydrogenated vegetable oil is well suited for the determination of the growth promoting properties of lactoflavin, provided it is supplemented with adequate amounts of vitamin B and the vitamin factor or group of factors contained in the rice polish concentrate. L.A.M.

ICE CREAM

28. Using Butter in Chocolate Coatings. H. A. SMALLFIELD, Dairy Dept., Ontario Agr. College, Guelph, Ont. Can. Dairy and Ice Cream J. *15*, 1, p. 24, Jan. 1936.

The results of investigations to improve chocolate coatings for ice cream bars are reported. When 35 per cent of the cocoa fat used for thinning the chocolate to the desired consistency for dipping the bars was replaced with butterfat there was a noticeable improvement in flavor and when 50 per cent was used there was a marked improvement. The presence of butterfat produced a somewhat more pliable coating, apparently did not affect the covering capacity of the coating but did increase the cost of the coating.

J.C.H.

29. Theory and Practice of Ice Cream Making. HUGO H. SOMMER. Second Edition, p. 639, 1935. Published by the author, Madison, Wisconsin.

This book gives a very clear and comprehensive presentation of the theoretical and practical phases of ice cream making. A discussion of every phase of the subject is given and the book is designed to appeal to the practical ice cream maker, short course and advanced students and research workers.

The second edition published three years after the first edition includes experimental data that have become available since that time. The arrangement of the subject matter has not been changed. The author states in the preface that, "It is gratifying that it has not been necessary to alter or abandon any of the theoretical explanations as advanced in the earlier edition."

A chapter on bacteriology has been added to the new edition. The subject is discussed as it pertains to ice cream and should be helpful in solving bacteriological problems that are encountered in the ice cream plant.

J.C.H.

30. **Suggestions for Making "Candy Flavoured Ice Cream."** C. A. IVERSON, Iowa State College, Ames, Iowa. Can. Dairy and Ice Cream J. *15*, 1, p. 55, 1936.

The author reports on methods of preparing candy for use in ice cream to impart a caramel and butterscotch flavor to the ice cream. J.C.H.

Other abstracts of interest are numbers 8, 21, 22, 23, 24, 25, 26, 27, 34, 35, 36, and 38.

MILK

31. **The Effect of Certain Ingested Fatty Oils Upon the Composition of Cow Milk Fat.** THOMAS PERCY HILDITCH AND HUBERT MORRIS THOMPSON, Dept. of Industrial Chemistry, Univ. of Liverpool, England. Biochem. J. *30*, p. 677, 1936.

It has been known for some time that the administration of cod-liver oil to lactating cows has a depressing effect upon the fat production, as well as other effects upon the physiology of the animal. It has been reported this effect was not observed when the unsaponifiable fraction of cod-liver oil was given in the ration, indicating that the injurious effect was possibly due to the component occurring as triglycerides.

The present investigation shows that some of the highly-unsaturated glycerides of cod-liver oil (but not its palmitoleic acid or the linolenic or linoleic acids of linseed oil) pass into milk fats; and it is suggested that selective absorption of these highly-unsaturated compounds by the enzymes responsible for the elaboration of typical cow milk fat retards their function and causes the depressant effects. K.G.W.

32. **The Effect of Ingested Cottonseed Meal Upon the Distribution of the Constituent Fatty Acids of Goat Milk.** R. W. RIEMENSCHNEIDER AND N. R. ELLIS, Animal Husb. Div., Bur. of Animal Ind. U. S. D. A. Washington, D. C. J. Biol. Chem. *114*, p. 441, 1936.

The fat of goat milk is generally acknowledged to contain greater amounts of capric, as well as caprylic and caproic acids, than does the fat of cow's milk. Certain objectionable qualities have been attributed to the increased amounts of these acids of lower molecular weight. In view of the probabilities of changing the properties of fats by the use of selected rations, as shown by experimental feeding of other animals, the authors investigated the effect of feeding cottonseed meal on the fat constants and presence of component fatty acids of the fat of lactating goats. Preliminary examinations of fat samples collected at the end of four experimental feeding

periods, each of 14 days' duration, indicated a lowering of the saponification number, Reichert-Meissel number, iodine number, water soluble acids, and thiocyanogen number, and an increase in the Polenske value. Partial recovery from the effects of feeding the cottonseed meal ration as the experimental periods progressed was indicated. A study of the fatty acid distribution by the methyl ester distillation method showed an increase in capric, lauric, myristic and stearic acids, chiefly at the expense of palmitic and oleic acids, when the cottonseed meal was fed. A slight lowering of butyric and caproic acids was observed. No evidence of linoleic acid was obtained, although acids of the arachidonic type were found. K.G.W.

33. An Apparatus for Milking Small Laboratory Animals and the Composition of Stock Rat Milk. WARREN M. COX, JR., AND ARTHUR J. MUELLER, Research Lab., Mead Johnson Co., Evansville, Indiana. J. Biol. Chem. *114*, Sci. Proc. XXX, XXII, 1936.

The authors in this abstract report the perfection of a micro-milking apparatus satisfactory for milking rats and guinea pigs. The percentage composition of rat milk at different stages of lactation was determined. The following figures are averages of all determinations: fat, 14.8 per cent; protein, 11.3 per cent; carbohydrate, 2.9 per cent; ash, 1.5 per cent; solids, 31.7 per cent; specific gravity, 1.047; ratio of lactalbumin to casein, 1:12; pH, 6.5 and 6.6. The extracted fat had a saponification number of 221 and an iodine number of 39.9. The size of the fat globules is approximately the same as in cow's milk. Buffer curves show 170 cc. of O.I/N acid (as compared to 65 cc. for cow's milk) to 100 cc. of rat milk are required to lower the pH to 4.0. K.G.W.

34. The Effect of Breed Characteristics and Stages of Lactation on the Vitamin C (ascorbic acid) Content of Cow's Milk. RUSSEL RASMUSSEN, N. B. GUERRANT, A. O. SHAW, R. C. WELCH AND S. I. BECHDEL. Penn. State College, State College, Pa. J. Nutrition *11*, p. 425, May, 1936.

The authors used the 2–6 dichlorophenolindophenol titration method to study the vitamin C content of cow's milk. Cows of the same breed, while receiving similar diets, were found to produce milks which showed wide variations in their ascorbic acid content. Such variations are thought to be due, in part at least, to differences in stages of lactation. Cows of different breeds, while receiving similar diets, produced milks which differed somewhat in their average ascorbic acid values. Of the five breeds studied, the Brown Swiss cows produced milks of the highest, and the Holstein cows produced milks of the lowest ascorbic acid value. Stage of lactation appeared to have a more definite effect upon the ascorbic acid content of milk than did breed differences. The ascorbic acid content of milk was found to be relatively high during the early stages of lactation, but decreased to

a minimum after about two months of lactation, and then increased to a maximum in the later stages of lactation. L.A.M.

35. The Influence of Milk Constituents on the Effectiveness of Vitamin D. G. C. Supplee, S. Ansbacher, R. C. Bender and G. E. Flanigan, Borden Co., Research Div., Bainbridge, N. Y. J. Biol. Chem. *114*, p. 95, 1936.

A number of studies have been conducted to ascertain the correlation between "potency" as numerically expressed in terms of "vitamin D rat units" and the clinical effectiveness of cod-liver oil, viosterol, milk from cows fed irradiated yeast, and irradiated milk. It has been recognized in some of these, that irradiated milk possesses a peculiar and marked clinical effectiveness. Various theories have been offered to explain the discrepancy between the empirical laboratory criterion and the clinical results obtained with irradiated milk. The presence of a "vital" factor not measureable by established procedures; a difference in the identity of the vitamin D as obtained from animal and plant sources; the inherent calcium and phosphorus, inherent cholesterol content of milk, and the prosthetic association of the lipid material with lactalbumin, have been suggested or studied as probably explanations for the clinical efficacy of irradiated milk over other vitamin D products.

The authors in this paper, have presented findings which show that certain milk constituents, particularly albumin, affect the response to administrations of vitamin D. Varying amounts of vitamin D in propylene glycol were added to two types of highly dispersed lactalbumin solutions, one consisting of substantially pure protein with prosthetically bound lipid material, the other consisting of the pure protein from which the lipid material was extracted by suitable solvents. Other highly dispersed solutions of lactalbumin and vitamin D were similarly prepared and the protein precipitated, filtered, repeatedly washed, and redispersed. These preparations were fed to properly prepared rachitic rats, the same amount of vitamin D in water being also prepared and administered for comparison. The results show that the healing response from the same amount of vitamin D is significantly intensified when the vitamin is ingested in association with the lactalbumin. The vitamin asociated with the colloid appears to be quantitatively precipitated up to certain limits since the filtrates from these solutions showed no anti-rachitic potency when fed at lower levels, and a greatly reduced potency when fed at higher levels.

When the vitamin D in proplyene glycol was appropriately added to various milks in predetermined amounts, results analagous to those obtained with lactalbumin, were obtained. Breast milk, which could not be activated to any substantial degree by activation, enhanced the effectiveness of the added vitamin, although not to as great a degree as did cow's milk. A

slightly greater effectiveness of standard vitamin D, diluted with olive oil, was observed when fed with milk; the increase in effectiveness was not as great as when the vitamin D was carried in a water miscible solvent. The authors conclude that since the vitamin carried by the lactalbumin showed a greater potency than when fed alone, it appears that the vitamin D and lactalbumin had formed a symplex. A symplex is a system consisting of a prosthetic group and a colloidal carrier of high molecular weight. In view of the experiments, the vitamin D would be the prosthetic group and the lactalbumin the carrier. Since, from the experiments, the lipid free protein vitamin combination induced a greater antirachitic response than the lipid vitamin combination, it is suggested that in the latter the vitamin is merely dissolved in the prosthetic group, namely, the lipid matter associated with the albumin. These observations are presented to explain the effectiveness of certain antirachitic preparations. K.G.W.

36. A Study of the Seasonal Variation of Vitamin D in Normal Cow's Milk. H. Ernest Bechtel and C. A. Hoppert, Mich. State College, East Lansing, Michigan. J. Nutrition *11*, p. 537, June, 1936.

A method is presented for the concentration of the antirachitic factors in milk fat by extraction with hot alcohol; thus making possible the biological assay of fats of low potency.

The monthly assay of milk fats from several sources, including samples from cows kept in dry lot as well as those pastured, over a period of two years showed that milk may vary as much as 900 per cent in antirachitic potency. Highest values were obtained during July, August, or September and lowest usually in February. Vitamin D values ranging from 4.8 to 43.8 U. S. P. units per quart of milk were observed in the case of Guernsey milk whereas the extreme values for Holstein milk were 3.1 to 27.7 U. S. P. units per quart. The data suggest that the cow has little or no opportunity to store vitamin D during lactation under ordinary dairy management conditions. The close correlation between the antirachitic potency of milk and the amount of available sunshine indicates that the exposure of cows to sunlight is a major factor contributing to the vitamin D content of milk. L.A.M.

37. New Zeland's Municipal Milk Supply. The Lancet *230*, p. 212, July 25, 1936.

Since 1922 the City of Wellington, the capital of New Zealand, has been operating a municipal milk supply. The milk is purchased from a cooperative dairy farmers' organization. The production area in the summer is 30 miles from the city, and in winter the collection area is extended to 60 miles. The milk is purchased by weight and averages 4.2 per cent butterfat.

Stringent hygienic conditions are enforced by city inspectors, and some

5000 laboratory examinations are made monthly. The milk is pasteurized at 145° F. for 30 minutes, cooled, and bottled. It is retailed in pint, pint and a half, and quart bottles, at an average retail price of slightly over 5 pence (10 cents in U. S.) per quart. About 85 per cent of the city's milk supply comes from the municipal plant, the remainder being sold by about 70 licensed farmers.

The net profit to the city for the year ending March 31, 1936 was 4376 pounds sterling, as against 6132 pounds sterling the previous year. The capital investment was 103,400 pounds sterling, which was raised by loan.

J.A.T.

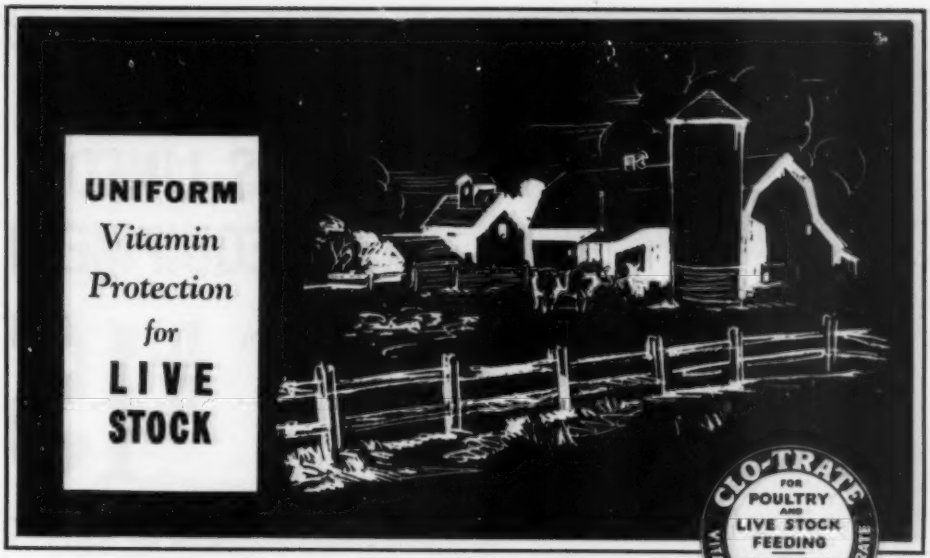
38. A Study of Alkalies for Bottle Washing. L. R. BRYANT (O. A. C. Guelph). Can. Dairy and Ice Cream J. *15*, 3 and 4, pp. 20 and 24, March and April, 1936.

The author's results showed the necessity of applying some form of sterilization treatment, such as chlorine sterilization, to the bottles after they leave the alkali soak tank to obtain consistently low bacterial counts. Recommendations were made for the use of a milder alkali with caustic soda for use in a soaker washer. It was found that one pound of sodium metasilicate, soda ash, or trisodium phosphate, can be used for this purpose to four or five pounds of caustic soda. The combination of caustic soda and sodium metasilicate in the series of experiments produced the cleanest appearing bottles of the three combinations. The addition of sodium metaphosphate to combinations of sodium metasilicate and caustic soda improved the shine. It was found that the total alkalinity of the soak tank solution in terms of caustic soda may be economically maintained between 1 and 2 per cent depending on conditions.

A quick and practical test for "total alkalinity" in terms of caustic soda was devised for frequent plant tests on the alkalinity in the soak tank.

J.C.H.

Other abstracts of interest and numbers 8, 9, 10, 11, 16, 21, 22, 23, 24, 25, 26, and 27.



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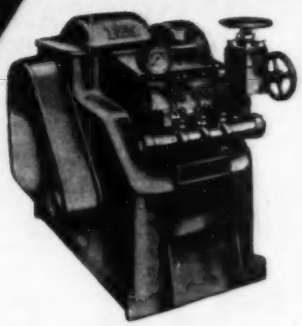

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Corroded equipment frequently causes metallic off-tastes and flavors. It is more difficult to clean . . . and this in turn leads to high bacteria counts. Although Diversol kills bacteria instantly . . . by more contact . . . it never rusts equipment. It is easy to use . . . dissolves instantly and completely . . . softens hardest water . . . is 100% stable.

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The difference between ordinary cleaning and the thorough cleanliness given you by Wyandotte can easily be determined by a very simple test,—you can actually *feel* it.

Ordinary cleaning leaves a grease film over which the thumb slides. Wyandotte-cleaned surfaces offer resistance when the thumb test is applied because no film is present,—the surface is truly clean.

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Bacto-Nutrient Agar

The Standard Medium for Plate Counts of Milk

Bacto-Nutrient Agar is recommended as the standard medium for the routine plate count of bacteria in milk. When it is prepared for use it conforms in every way to the medium described in "Standard Methods of Milk Analysis" of the American Public Health Association. It requires no further adjustment of its reaction or filtration of the solution, and is ready for use immediately after sterilization. After the usual autoclave sterilization the medium has a reaction of pH 6.7.

Plate counts of the bacteria in milk and other dairy products obtained on this medium are reliable and accurate because the medium is always uniform. The use of Bacto-Nutrient Agar for milk counts is allowable in the "Standard Methods" procedures.

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